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CALCIUM AND PHOSPHORUS METABOLISM IN MAN AND ANIMALS WITH SPECIAL REFERENCE TO PREGNANCY AND LACTATION

BY

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Conference Chairman
FRANKLIN C. McLEAN

Organizing Chairman and Consulting Editor
ROBERT R. MARSHAK

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* This series of papers is the result of a conference on *Calcium and Phosphorus Metabolism in Man and Animals with Special Reference to Pregnancy and Lactation* held by The New York Academy of Sciences, Section of Biology, January 10 and 11, 1956.

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Part I. Calcium Balance and Turnover Studies

RADIOCALCIUM STUDIES IN PREGNANCY

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Introduction

This paper reviews the contributions that radioisotopes have made to an understanding of the physiology of calcium metabolism in pregnancy. Because the interests of the conference on which this monograph is based extend to both man and animals, attention is given primarily to man, bovines, and the rat. Studies with small laboratory animals have been most numerous. They offer the particular advantage that total fetuses and dams can be analyzed to give values for net transfer of substances. Sufficient numbers of animals can be used, also, to provide reasonable statistics, and steady-state radioisotope experiments can be done. Cattle as experimental subjects offer advantages in that more detailed studies are possible on tissue and skeletal deposition. Furthermore, data are needed because of the economic importance of this species. Radioisotope studies of human pregnancy have not been done, as far as is known. Here the problem is to determine the extent to which basic principles gained from observations on other species can be applied in interpretation of the vast fund of clinical knowledge.

The techniques of radioisotope usage are now well documented and will not be discussed further.[1-7] The main advantages of tracers are that they can provide (1) determination of rate of ion movement across the placenta; (2) estimation of relative contribution of maternal stores and diet to the fetus; (3) visualization of skeletal deposition; and (4) an over-all concept of ion movement within and between the mother and fetus.

General Absorption and Excretion

Before discussing studies of pregnancy brief mention should be made of factors involved in absorption and excretion, with some emphasis on a summation of data from cattle. Obviously, the drain on the maternal skeleton to satisfy the developing fetus will be governed by the extent to which calcium is absorbed from the diet and utilized. The excellent review of Nicolaysen, Eeg-Larsen, and Malm[8] should be consulted for reference to the original literature on this aspect of the physiology of calcium metabolism.

Fecal endogenous loss. The term "fecal endogenous" is applied to the calcium that is transferred from the blood to the intestinal canal and subsequently excreted. The fecal endogenous loss can be used to calculate the net digestibility of calcium from ordinary balance studies as indicated in TABLE 1. Modifications of the isotope dilution method for measurement of fecal endogenous losses have been described by Hevesy[4] for phosphorus in man; by Kleiber and his co-workers[12, 13] for phosphorus in livestock; by Comar *et al.*[14] and Visek

TABLE 1

TYPICAL FECAL ENDOGENOUS LOSSES AND DIGESTIBILITIES OF CALCIUM IN MAN, CATTLE, AND RATS

	Calcium, gm./day		Fecal endogenous Ca		Digestibility, per cent	
	Intake	Feces	gm./day	mg./day/kg body wt.	Apparent*	Net†
Man[9]						
Patient B	0.529	0.408	0.091	(1–2)‡	23	41
Patient L	0.638	0.278	0.067		56	69
Cattle[10]						
0.3 months old	8.8	0.6	0.41	12	93	98
1 month old	7.4	1.2	0.97	14	84	98
6 months old	21	16	3.3	18	24	41
25–34 months old	27	22	5.4	17	17	36
aged	19	24	9.2	21	−28	22
Rats[11]						
3 months old	0.024	0.0085	0.004	23	65	86

* Apparent digestibility $= \dfrac{\text{Calcium intake—fecal calcium}}{\text{Calcium intake}} \times 100.$

† Net digestibility $= \dfrac{\text{Calcium intake—fecal calcium} + \text{fecal end. calcium}}{\text{Calcium intake}} \times 100.$

‡ Estimated value; body weights not available.

et al.[15] for calcium in cattle; by Blau *et al.*[16] for calcium in man; and have been reviewed by Comar and Wasserman.[17]

TABLE 1 presents some typical values for calcium balance and fecal endogenous losses. Of interest are the following observations: (1) The absorption of calcium decreased with increasing age. (2) In cattle the fecal endogenous loss was closely correlated with body weight, although the endogenous loss per unit of body weight tended to increase slightly with age. (3) In man, the fecal endogenous loss per unit of body weight was smaller than for cattle or rats.

Many factors affect the digestibility of calcium. Some of the conditions or substances that promote calcium absorption include (1) vitamin D; (2) specific substances in milk such as lactose, proteins, peptides, and amino acids;[18] and (3) undernourishment with calcium, which will be discussed in a later section. Depressive effects have been attributed to: (1) high phosphate concentrations, (2) phytic acid, (3) faulty fat absorption, (4) oxalic acid, and (5) emotional disturbances.

Analysis of data from experiments with 8 pregnant cows indicated that pregnancy itself did not significantly increase the absorption of calcium.[19]

Urinary excretion of calcium in man and cattle. TABLE 2 presents a summary of urinary excretions of calcium. The following general conclusions for man have been compiled by Nicolaysen *et al.*[8]

The quantity of calcium in the urine is dependent on endogenous factors and on calcium intake per unit of weight. In general, the urinary calcium at an intake of calcium of 10 mg. Ca per kg. body weight averaged 25 per cent of the intake with minimum and maximum values of 5 and 60 per cent, respectively. TABLE 2 shows that short-term halving of the calcium intake caused only a

TABLE 2

URINARY EXCRETION OF CALCIUM BY MAN AND CATTLE

	Age	No. observed	Ca intake, gm./day	Ca in urine	
				gm./day	Per cent of intake
Women[8]	40–79 yr.	109	0.785	0.160	20.4
Men[8]	40–79	16	0.917	0.231	25.1
Men[8]	20–76	30*	0.900	0.230	25.6
Men[8]	20–76	15*	0.450	0.207	46.1
Cattle[19]	20–32 months	8	25	0.289	1.2

* Same individuals, showing effects of halving the calcium intake.

small decrease in urinary calcium. Of particular interest was the constancy of the urinary calcium of individuals. Effects on urinary calcium excretion have been caused by extra ingestion of phosphate, by certain hormones, and by immobilization.

The main feature in cattle is the small urinary excretion. In most studies the urinary excretion of calcium by cattle can be neglected for practical purposes. This also means that the bovine is unable to provide for body conservation of calcium by the reduction of normal urinary excretion.

Adaptation. In general, under conditions of low calcium intake, the body is able to adapt primarily by increasing the efficiency of absorption. It is not yet possible to ascertain the extent of any decrease in the calcium secretion of the digestive juices, for although the fecal endogenous loss may be reduced, most of this reduction may reflect increased absorption. Decrease in urinary calcium may also contribute to conservation by the body. Of particular importance is the length of time and severity of depletion required for adaptation to occur.

Nicolaysen *et al.*[8] have shown that loss of 5 per cent of the body calcium in rats is followed by a distinct compensatory increase in the efficiency of absorption. When the calcium level of the diet was as low as 0.04 per cent, the decrease in fecal excretion was demonstrated in a matter of weeks. Results were equivocal on a diet of 0.25 per cent calcium. It appeared that there was a considerable delay in compensation when the calcium deprivation was not severe.

TABLE 3 presents some data for man and cattle. The values for man, from Laszlo *et al.*,[9] indicate that considerable increase in calcium intake over one-month periods did not change appreciably the urinary or fecal endogenous losses. Nicolaysen *et al.*[8] have reported long-term studies with 20- to 76-year-old men who were in balance with a daily intake of 900 mg. of calcium and were reduced to a 450 mg. level. In general, adaptation to a new balance required from 2 to 8 months. The cattle data in TABLE 3 indicate that animals on a low-normal calcium intake for 6 months showed no differences in fecal endogenous loss. After 15 months the animals had apparently adapted as shown by about a 30 per cent reduction in fecal endogenous calcium.

Behavior of Ca^{45} *in the body.* The excretion of Ca^{45} by man and cattle is

Table 3

Illustration of Time Factor in Adaptation to Dietary Calcium Levels

	Time on diet	Ca intake, gm./day	Urinary Ca, gm./day	Fecal end. Ca, gm./day
Patient B[9]		0.107	0.090	0.091
	1 month	0.529	0.085	0.091
	1 month	1.446	0.103	0.073
Cattle*[20]	6 months	10	—	2.44
	6 months	30	—	2.52
	15 months	20	—	2.55
	15 months	48	—	3.79

* Six paired animals started on experiment; one group placed on low normal calcium intake, the other on high normal; fecal endogenous loss determined after 6 and 15 months on experiment.

Table 4

Typical Values for Excretion of Radiocalcium by Man and Cattle

	Age	Ingested Ca^{45}*		Parenteral Ca^{45}		Removal rate from blood†
		Feces	Urine	Feces	Urine	
Man[16]	Adult	53	6	11	10	
Cattle[21]	0.3 months	3.0	<0.5	2.0	<0.5	102
	1	6.5	<0.5	1.0	<0.5	
	6	62	<0.5	8.0	<0.5	72
	25–34	70	<0.5	15	<0.5	26
	Aged	83	<0.5	25	<0.5	

* Values in percentage of administered dose.
† Percentage of the calcium in plasma transferred per minute from plasma.

summarized in table 4. These values reflect the behavior already discussed for over-all calcium handling.

The details of Ca^{45} movement have been described in several papers.[21, 22, 23] It has been shown that the rate of exchange of stable calcium ions between blood and other compartments is quite rapid, ranging from about 26 per cent of the total blood calcium per minute in aged cattle to over 100 per cent per minute in young calves. Thus the circulating calcium atoms are continuously and rapidly being removed from the blood to be replaced primarily by calcium atoms from the skeleton or gastrointestinal tract. One has the impression that the calcium ions in various anatomical pools are easily interchangeable; *e.g.*, plasma, extravascular fluid, soft tissues, and exchangeable portion of bone. Nonexchangeable pools that have recently been suggested include colostrum[24] and possibly a fraction in the udder of the milking cow.[25] As the animal becomes older, less of the bone calcium is available for exchange with blood calcium.

Shortly after Ca^{45} enters the blood stream, either by injection or absorption, most of it will become sequestered in the skeleton within a matter of minutes. Small amounts will be excreted, as already shown, and a low level will be maintained in the blood and soft tissues. The radioactivity deposited in bone will for the most part remain in the original location until this region undergoes resorption.

Transfer of Ions Across the Placenta

Methods. The classical methods for quantitative estimation of the rate of placental transfer can be illustrated by studies with inorganic phosphate.[26] The fundamental equation is:

$$\frac{P^*_{mp}}{P_{mp}} = \frac{P^*_f}{P_f} \qquad (1)$$

where P^*_{mp} = concentration of P^{32} present as inorganic phosphate in maternal plasma

P_{mp} = concentration of inorganic phosphate in maternal plasma

P^*_f = amount of P^{32} transferred to fetus in the experimental time period

P_f = amount of phosphate transferred to fetus in experimental time period

The basic assumption is that the radioisotope and its stable counterpart cross the placenta in the same proportion as they occur in the fluid bathing the membrane. Experience would indicate little if any difference in movement of isotopes of mass as great as that of phosphorus. Also, EQUATION 1 is valid only if none of the radioisotope transferred to the fetus is lost from the fetus during the experimental period. Experiments with the guinea pig demonstrated that loss of P^{32} from the fetus was insignificant during 30 to 45 minutes after administration of the radioisotope. It was also necessary to show that practically no P^{32} in the plasma becomes organically bound during this short period.

Further understanding of the method can be gained from consideration of the estimation of the terms in EQUATION 1.

P^*_{mp}. After intravenous injection of P^{32} the plasma concentration follows the typical steep disappearance curve. The average value of plasma concentration between injection and killing of the mother must be determined. This was estimated by establishing a disappearance curve for animals similar to those to be employed. The average concentration for a given animal killed at some certain time was then determined by measurement of the corresponding area under the standard curve, and application of a correction factor to the plasma concentration observed at the end of the experiment.

P_{mp}. This quantity was determined by the usual methods for plasma inorganic phosphorus.

P^*_f. This quantity was determined by radioassay of the fetus according to usual methods.

A typical experiment that illustrates the use of this method is given in TABLE 5. Some general conclusions from a series of determinations were as follows. Inorganic phosphate was supplied to the fetuses in about the quantities required for growth. For example, it was shown by the P^{32} method that a 105 gm. fetus received 1.8 mg. phosphorus per hour as compared with an actual phosphorus retention in growth of 1.5 to 2.2 mg. per hour. This is unlike sodium and water, which are supplied to the fetus in amounts that are 50 to several hundred times those incorporated in growth. It is also of interest to note that

TABLE 5

TYPICAL VALUES FOR ESTIMATION OF PLACENTAL TRANSFER RATE OF INORGANIC PHOSPHATE IN THE GUINEA PIG[26]

Fetal weight	52.8 gm.
Delivery time	35.3 min.
Placental weight	3.5 gm.
Observed P^{32} in maternal plasma	263 beta cts./sec./cc.
P^*_{mp}	453 beta cts./sec./cc.
P^*_f	3800 beta cts./sec.
P_{mp}	4.42 mg. inorganic P/100 cc.
P_f (by calculation)	0.18 mg. P/hr./gm. of placenta

TABLE 6

ILLUSTRATION OF RAPIDITY OF CALCIUM TRANSFER TO THE RAT FETUS*[27]

Time after ingestion of Ca^{45} by dam*, hrs.	Per cent of Ca^{45} dose	
	Body of dam†	Fetuses
0.5	49	14
1	61	24
5	51	24

* Dams in 19th to 22nd day of gestation.
† Less digestive tract.

the phosphorus retained per hour by the fetuses was about four times the total inorganic phosphorus of the maternal plasma. This situation will be discussed in more detail in terms of calcium.

Placental transfer rate in the rat. Quantitative estimations of rate similar to those described for phosphate have not been reported for calcium as far as is known. The same procedures, however, would be applicable. An indication of the rapidity of the transport of calcium is available from the thesis of Plumlee[19] and the data shown in TABLE 6. It will be noted that within 30 minutes after oral administration approximately 63 per cent of the Ca^{45} had been absorbed by the mother, and 14 per cent of the dose was already transferred to the fetuses. This demonstrates both the rapid absorption from the digestive tract and the speed of placental transport. Within 1 hour after administration the fetuses had already incorporated the maximum quantity of the radioisotope.

Placental transfer rate in cattle. Studies on cattle have been reported by Plumlee[19] and Plumlee, Hansard, Comar, and Beeson.[28] It was not practical to determine total amounts of Ca^{45} transferred to the fetus, so that only an idea of the transfer rate is available from concentration values. The time pattern of calcium transfer was shown by experiments in which 8-month pregnant heifers were killed at varying times after intravenous injection of radiocalcium.

TABLE 7 presents the comparative concentrations in the muscle (representative of soft tissues) and typical bone regions of the fetus and dam at 10 minutes, 8 hours, 30 hours, and 7 days after injection. The avidity of the fetal bone for calcium is demonstrated by comparing the concentrations in the bones of the fetus and dam at 10 minutes, in terms of the Ca^{45} levels of the circulating fluids to which bones were exposed during that 10 minutes. The bone values

Table 7

Comparative Concentrations of Ca^{45} in Heifers and 8-Month Fetuses at Varying Times after Intravenous Injection[28]

Time after injection	10 min.	8 hrs.	30 hrs.	7 days
G. muscle				
Fetus	2.5*	4.8	2.8	2.9
Dam	28	9.8	6.2	1.2
Metatarsal shaft				
Fetus	10	180	410	2100
Dam	11	130	73	250
Metatarsal epiphysis				
Fetus	16	320	550	1100
Dam	42	130	200	290

* Values expressed as per cent of dose × 10^5 per gm. fresh tissue.

of the dam varied from 1 to 6 times higher, yet the circulating fluid value of the dam must have averaged considerably more than 10 times higher than that of the fetus, as judged from comparative blood values shown in FIGURE 1. As expected, the bone values increased with time after injection, and the soft tissue values generally decreased.

FIGURE 1 shows the comparative specific activities of calcium in the blood and muscle of the dam and fetus as related to time after injection. The fetal blood level at 10 minutes shows that the labeled calcium passed into the fetal circulation at a relatively rapid rate. Of particular interest was the fact that the specific activity of the fetal blood was still considerably lower than that of the maternal blood at 8 hours. The fetal muscle value, also, was still lower than the specific activity of the fetal blood. A satisfactory explanation is not yet available for these observations. TABLE 8 gives the average values for soft tissue and bone expressed as ratios of fetal to maternal specific activities. As expected, the soft tissues approach a value of 1 at 7 days while the bone reaches a value of 10, indicating the increased accumulation that occurs in the fetal bone. Again, the high values for bone as compared to soft tissue emphasize the ability of fetal bone to retain the circulating radiocalcium.

Transport of Calcium in Relation to Fetal Age

The gross rates of calcium accumulation in the developing fetus can be gained from chemical analysis at different fetal ages. Some typical growth and calcium accumulation data for man, cattle, and the rat are presented in TABLE 9. Calcium values for man and cattle are plotted on a semilogarithmic scale in FIGURE 2.

It may first be noted that little calcium is deposited in the fetus during the first trimester of pregnancy and that the greatest accumulation occurs during the last trimester. The slope of the curve for cattle is slightly greater than that for man, indicating a faster rate of increase for cattle. It appears that a decrease in slope occurs for man at 5 to 6 months and for cattle at about 8 months. When values for the rat are plotted in a similar way, it is apparent that the slope of this curve is much steeper than for man and cattle, and that an inflection occurs with a decrease in slope at about 19 days.

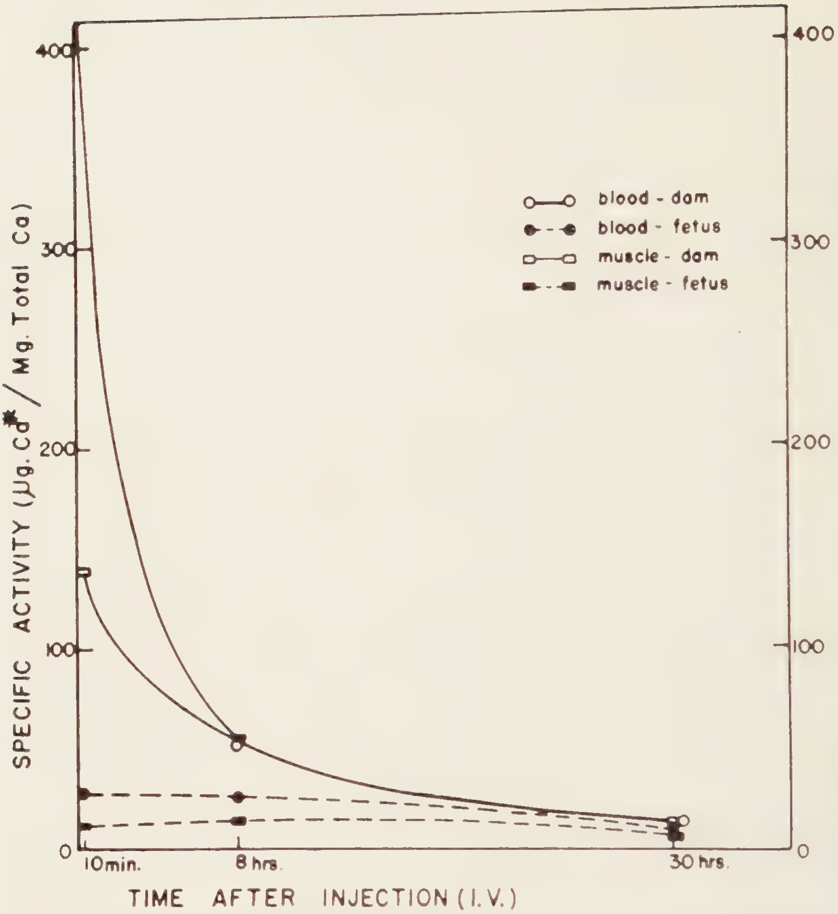

FIGURE 1. Specific activity of calcium in blood and muscle of dams and 8-month fetuses at varying times after intravenous injection of labeled calcium into the dam.[28]

Studies with cattle. Experiments have been described in which Ca^{45} was given orally to heifers at varying stages of pregnancy. The animals were killed at 7 days after dosage for determination of concentrations and specific activities of radiocalcium in the tissues of the dam and fetus.[19, 28] TABLE 10 presents the comparative concentrations in the muscle (representative of soft tissues) and typical bone regions of the fetus and dam killed at 7 days after oral dosage; fetal ages were 3½, 6, 7½, and 8½ months. In general there were no large effects of fetal age on the concentrations in the soft tissues.

The effects of fetal age on appearance of Ca^{45} in the bone are of interest and are therefore presented graphically in FIGURE 3. It is noted that in the 3½-month-old fetuses more Ca^{45} appeared in the shafts than in the epiphyses. This situation was reversed at 6 and 7½ months. Of particular significance is the fact that all bone samples showed a peak of accumulation between the 4th

TABLE 8

RATIO OF FETAL SPECIFIC ACTIVITY TO DAM SPECIFIC ACTIVITY IN HEIFERS AND 8-MONTH FETUSES AT VARYING TIMES AFTER INTRAVENOUS INJECTION[28]

Time after injection	10 min.	8 hrs.	30 hrs.	7 days
Soft tissues*	0.08	0.42	0.56	1.05
Bone†	0.56	2.9	4.9	10

* Average of blood, liver, kidney, spleen, gastrocnemius muscle.
† Average of mandible, vertebra, metatarsal shaft and epiphysis, rib shaft and epiphysis.

TABLE 9

CALCIUM ACCRETION IN THE DEVELOPING FETUS OF MAN, CATTLE, AND THE RAT

Man[29]			Cattle[30]			Rat*[27]		
Age months	Wt. gm.	Ca, gm.	Age, months	Wt., kg.	Ca, gm.	Age, days	Wt., gm.	Ca, mgm.
1	0.04	—	5	2.56	10.1	14	0.10	0.018
2	3.0	0.032	6	6.505	43.2	15	0.14	0.042
3	36.0	0.25	7	16.46	140	16	0.25	0.088
4	120.0	1.1	8	31.8	375	17	0.62	0.46
5	330.0	3.3	9	49.3	673	18	1.5	1.5
6	600.0	7.5				19	2.7	5.2
7	1000.0	11.6				20	3.8	8.1
8	1500.0	20.4				21	4.9	8.7
9	2200.0 3200.0	—				22	5.6	12.3

* Per fetus.

and 7th months. Since the values for plasma and soft tissue did not show a similar increase, it is assumed that this behavior was characteristic of the bone metabolism.

TABLE 11 and FIGURE 4 show the specific activities as a function of fetal age. For all fetal ages, a higher turnover rate was shown (FIGURE 4) in the epiphyseal regions than in the shaft areas. The decrease in specific activity of bone with increased fetal age was primarily accounted for by the fact that the normal calcium content of the fetal bone increases with age. During the 7 days between dosage and killing more stable calcium was laid down in the older fetuses.

Deposition in bone. FIGURE 5 presents autoradiograms of the metatarsals of a heifer and 6-month fetus killed at 7 days after ingestion of Ca^{45}. FIGURE 6 presents autoradiograms of the femur and metatarsal of a lamb whose mother was injected with Ca^{45} about 3 weeks prior to parturition. The light gray areas represent the new bone formed between time of dosage and death. The interpretations of such bone autoradiograms have been discussed elsewhere.[2, 23]

Studies with laboratory animals. A quantitative relationship between the transfer rate of a mineral and fetal age can best be illustrated by data on phosphate transfer in the guinea pig measured as previously described.[26] It is clear from TABLE 12 that phosphate was supplied to the fetus at a rate directly related to the size and need of the fetus.

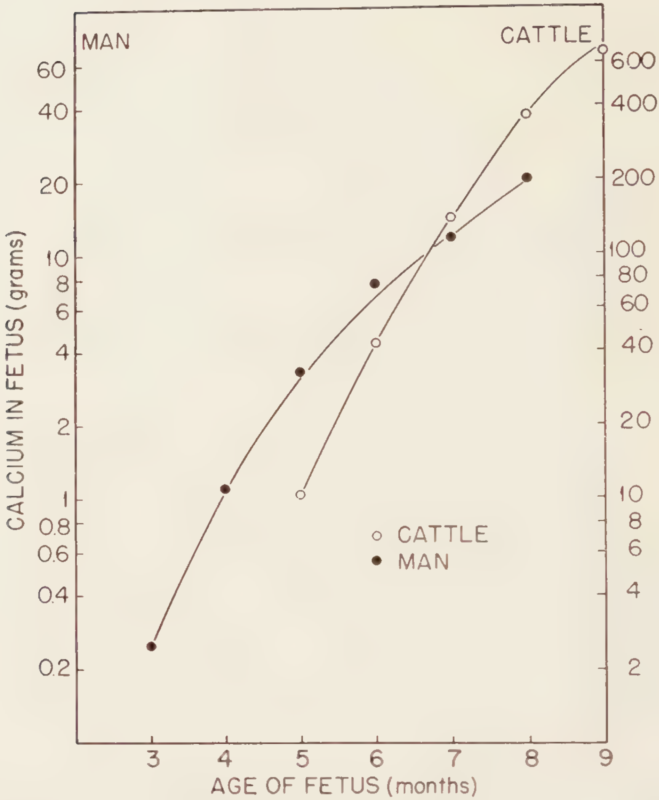

FIGURE 2. Accumulation of calcium in the developing fetus of man and cattle.

Contribution of Maternal Skeleton and Diet to Fetal Calcium

Early work with radiocalcium by Pecher and Pecher[31] demonstrated two facts: (1) The maternal skeleton does supply calcium to the developing fetus. (2) The last calcium fixed in the mother's bone is the first to be removed for transfer to the fetus. These findings have been confirmed many times and are based on studies in which radiocalcium is given to the female before conception or before calcification has started in the fetus, so that the radiocalcium becomes incorporated in the mother's bones. The bones of the newborn fetus show a higher specific activity (Ca* Ca) than does the skeleton of the mother. This means that some of the last calcium laid down in the mother's bones (labeled with Ca^{45} before conception) was mobilized and transferred to the fetus preferentially as compared with the rest of the maternal calcium.

Time relationships. It is of interest to consider the rate of calcium accumulation in the fetus as compared with the amount of calcium present in the blood, which serves as the direct source. Some estimates of these quantities are presented in TABLE 13. It is noted that each hour the human fetus drains about 7 per cent of the blood calcium, the bovine fetus about 50 per cent, and the rat

TABLE 10

COMPARATIVE CONCENTRATIONS OF Ca^{45} IN HEIFERS AND FETUSES OF VARYING AGES AT 7 DAYS AFTER ORAL DOSAGE[28]

Age of fetus	3½ months	6 months	7½ months	8½ months
G. muscle				
Fetus	1.1*	1.0	0.54	0.32
Dam	0.40	0.55	0.51	0.37
Metatarsal shaft				
Fetus	840	820	350	290
Dam	79	85	49	45
Metatarsal epiphysis				
Fetus	350	1300	580	250
Dam	120	86	71	61

* Values expressed as per cent of dose × 10^5 per gm. fresh tissue.

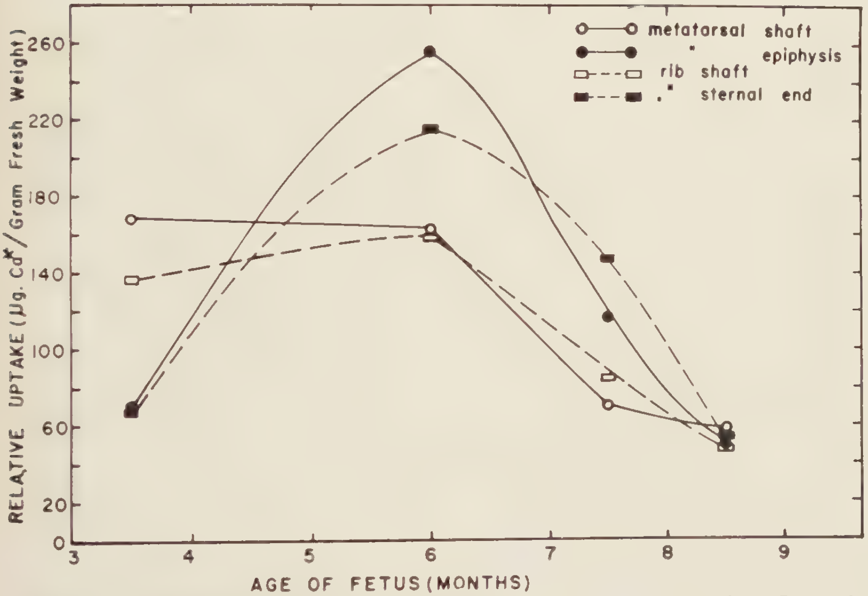

FIGURE 3. Concentration of labeled calcium in bones of bovine fetus as function of fetal age; Ca^{45} was ingested by dam 7 days before killing.

fetus about 100 per cent. If we assume that fetal growth is continuous, then it is clear that the blood calcium must be almost continuously replenished. This requirement is most severe for the rat and least severe for man. Even in man, however, a four-hour period of no replenishment of blood calcium would cause about a 70 per cent drop in blood calcium.

It is suggested that the periodicity of feeding and rate of absorption will govern to a large extent whether the fetal calcium is being derived from the maternal skeleton, or whether it is coming directly from calcium absorbed into the blood from the digestive tract. For example, if the digestive tract contains little or no food calcium, then presumably the homeostatic mechanism operates

TABLE 11

RATIO OF FETAL SPECIFIC ACTIVITY TO DAM SPECIFIC ACTIVITY IN HEIFERS AND THEIR FETUSES OF VARYING AGES AT 7 DAYS AFTER ORAL DOSAGE[25]

Age of fetus	3½ months	6 months	7½ months	8½ months
Soft tissue*.	0.92	1.2	1.5	0.81
Bone†.	13	11	7	5.9

* Average of blood, liver, kidney, spleen, G. muscle.
† Average of mandible, vertebra, metatarsal shaft and epiphysis, rib shaft and epiphysis

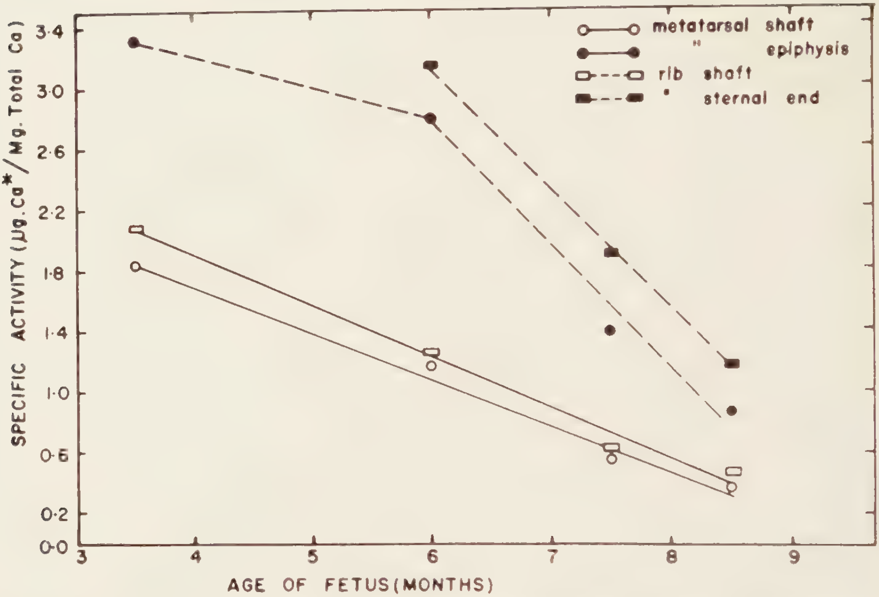

FIGURE 4. Specific activity of calcium in bones of bovine fetus as function of fetal age; Ca^{45} was ingested by dam 7 days before killing.

to mobilize calcium from the maternal skeleton and thus maintain the blood level. During such a period practically all the calcium being laid down in the fetus would be derived from the maternal skeleton. On the other hand, shortly after feeding, enough calcium would enter the blood from the gastrointestinal tract to satisfy the fetal demands. During this time period practically all the calcium being transported to the fetus would be directly derived from the diet. Also, the excess calcium entering the blood could be deposited in the skeleton for later withdrawal. Although there is no direct evidence for this kind of cycle in mammals, it has recently been demonstrated in the formation of the egg shell in birds.[32]

Measurement of maternal and dietary contribution. Radioisotope procedures are usually well suited for determination of the relative contribution of 2 sources to a product.[2] The general procedure, as illustrated in FIGURE 7, consists first of labeling one of the sources; then, the specific activity of the

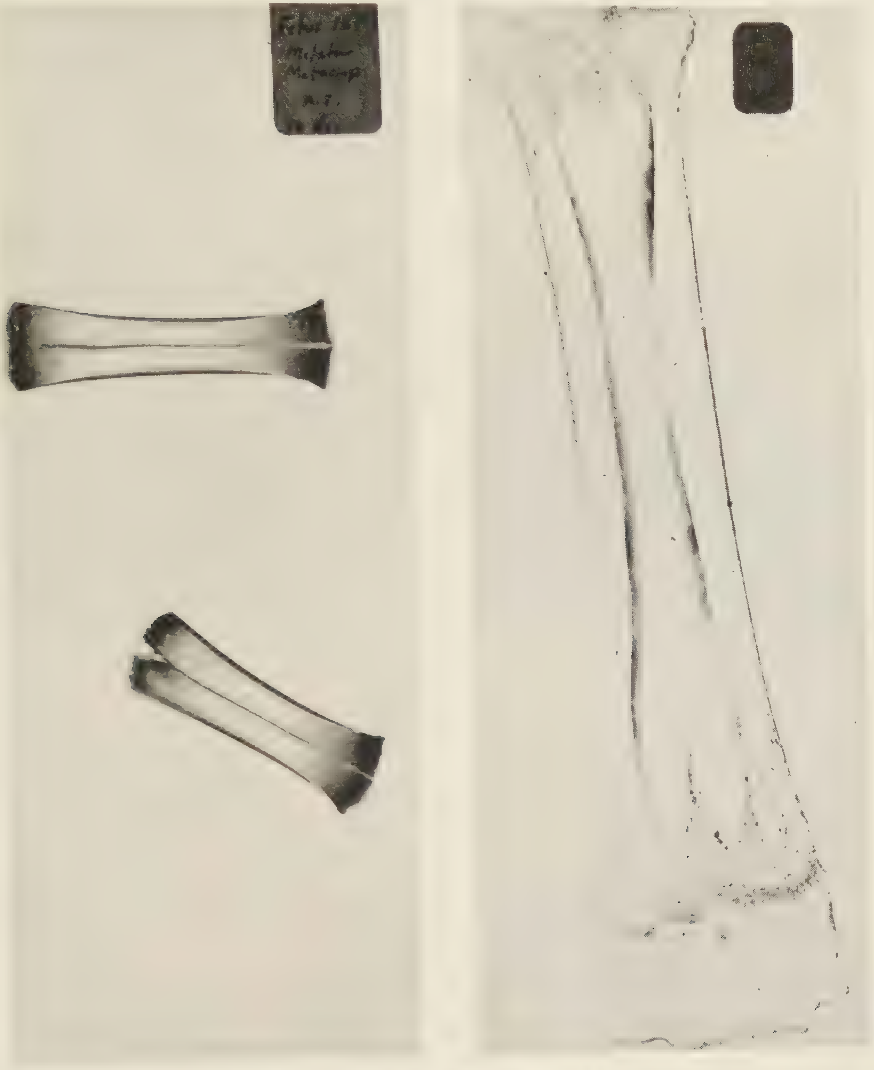

FIGURE 5. Autoradiograms of metatarsals of heifer and 6 months fetus killed 7 days after oral administration of Ca^{45}.

product (fetus) divided by the specific activity of the labeled source (diet) is the fraction of the product calcium that came from the labeled source.

In fetal growth, however, a difficulty arises because of the transfer of some dietary calcium to the maternal skeleton during the gestation period. This is represented by the dotted arrows in FIGURE 7. The same problem arises even if the skeleton of the dam is labeled instead of the diet. This is because some of the unlabeled calcium from the diet will be laid down in the maternal skeleton

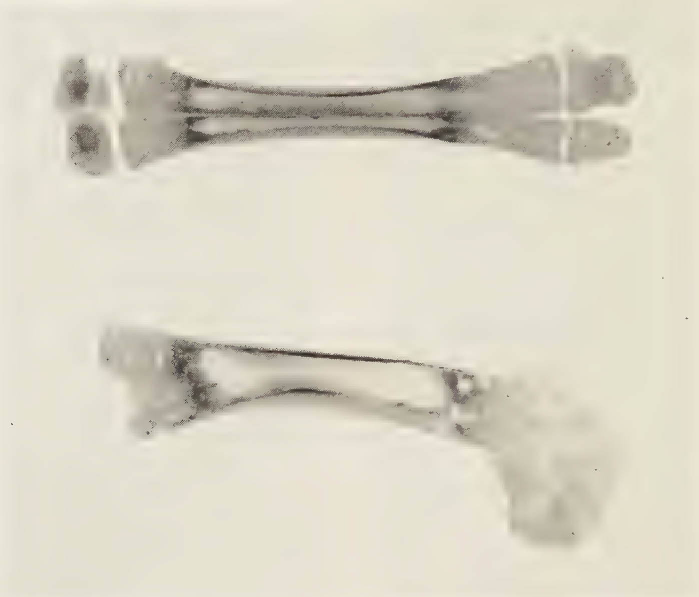

FIGURE 6. Autoradiograms of femur and metatarsal of lamb whose mother received Ca^{45} about 3 weeks prior to parturition.

TABLE 12

TRANSFER OF INORGANIC PHOSPHATE TO THE GUINEA PIG FETUS AS A FUNCTION OF FETAL WEIGHT[26]

Fetal wt., gm.	Inorganic phosphate supplied to fetus, mg./hr.*	Total phosphate retained in growth, mg./hr.†
34	0.4	0.4–0.5
63	0.9	0.7–1.0
105	1.8	1.5–2.2

* Measured by P^{32} method.
† Estimated by chemical analysis of fetuses of different ages.

TABLE 13

ESTIMATED VALUES OF RATE OF CALCIUM ACCUMULATION IN FETUS DURING LATTER TERM OF GESTATION, AND AMOUNT OF CALCIUM IN TOTAL BLOOD OF MOTHER

	Calcium accumulation, mg./hr.	Calcium present in blood, mg.
Human fetus	17	250
Cattle fetus	1300	2000
Rat fetus	0.5	0.6

(thus reducing the skeletal specific activity), and will then be preferentially mobilized for transfer to the fetus. Thus the ratio of specific activities gives information concerning: (1) how much of the fetal calcium was derived from calcium present in the maternal skeleton before the experiment started, and (2) how much of the fetal calcium was derived from calcium atoms originating in the diet. It is not possible to determine by this method how much of the fetal calcium had at any time been in the maternal skeleton.

A study with radiostrontium, designed for other purposes, has provided information of this sort for the rat.[33] In this work the maternal skeleton was completely labeled with Sr^{90} by growing the animals so that their entire skeleton was formed from a diet of a constant strontium-90 to calcium ratio. Certain correction factors had to be applied to account for the differential utilization of calcium and strontium in bone formation; the original paper may be consulted for details. It was estimated that under the conditions of the experiment, about 30 per cent of the fetal calcium had been derived from calcium that had been present in the mother's skeleton before conception.

Summary

The studies described give rise to at least a partial picture of calcium ion movement in the mother and fetus. These ideas will be summarized in terms of the pathway of calcium atoms entering the blood. It must be remembered, however, that the movement of these specific atoms sometimes represents (1) the net transfer of calcium (as in new bone formation) or (2) an exchange process in which the specific atoms are replaced by those from another pool so that no net transfer of calcium occurs (as in exchange of ions between the plasma and extravascular fluid).

FIGURE 8 presents a schematic illustration of calcium flow in the mother

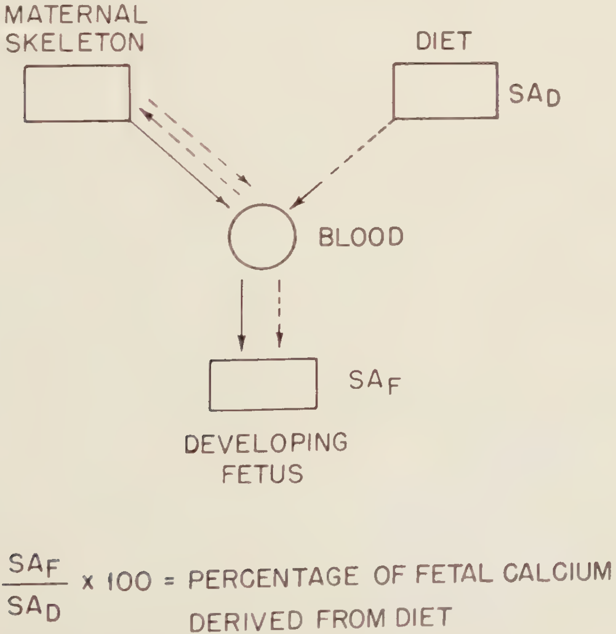
MATERNAL SKELETON
DIET
SA_D
BLOOD
SA_F
DEVELOPING FETUS
$\frac{SA_F}{SA_D}$ x 100 = PERCENTAGE OF FETAL CALCIUM DERIVED FROM DIET

FIGURE 7. Illustration of method for estimation of contribution of maternal and dietary calcium to the developing fetus.

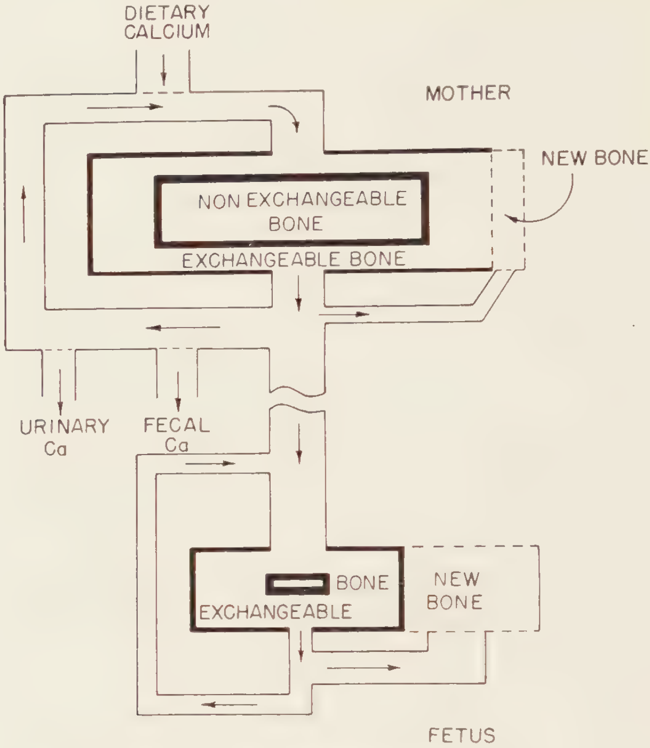

FIGURE 8. Schematic flow diagram of calcium in mother and fetus.

and developing fetus. When calcium atoms are absorbed from the digestive tract and enter the blood, they are rapidly removed by several competing processes: (1) mixing with the body fluids and deposition in the soft tissues of the mother; (2) deposition in the bones of the mother; (3) excretion into the gastrointestinal tract and the urine; and (4) transfer across the placenta with subsequent entry into fetal body fluids, soft tissues, and bones.

The outstanding features are (1) the interchangeability of calcium ions between the various pools; (2) the rapidity of transfer across the placental barrier; (3) the avidity of the fetal bones for calcium; (4) the high rate of transfer of calcium across the placenta as compared with the amount of calcium present in the blood and the need for constancy of the calcium level in the maternal blood; and (5) possible short-term cyclic effects related to the schedule of feeding and rates of absorption.

The question may well be raised concerning the mechanism of transport of calcium across the placenta. Undoubtedly the fetal requirement is the governing factor, since it is well known that the calcium value of the maternal serum may fall during the latter term of pregnancy. A most straightforward explanation would be that the calcium level of the fetal plasma is lowered by the drain of fetal bone formation, and that calcium moves from the maternal to fetal blood in response to a concentration gradient. This mechanism must be ruled out, however, because it has been shown that the fetal plasma calcium (even the ionized calcium) is higher than the maternal plasma calcium.[29] This leads to the conclusion that "active transport" is probably involved, a virgin field for investigation. Of possible interest for future work is the early observation that the placenta is characterized by a high arginine content,[29] and the recent observation that arginine and lysine are quite effective in promoting calcium absorption from the digestive tract.[18]

References

1. Calvin, M., C. Heidelberger, J. C. Reid, B. M. Tolbert & P. F. Yankwich. 1949. Isotopic Carbon. John Wiley & Sons. New York, N. Y.
2. Comar, C. L. 1955. Radioisotopes in Biology and Agriculture, Principles and Practice. McGraw-Hill. New York, N. Y.
3. Comar, C. L., S. L. Hood, S. L. Hansard, M. P. Plumlee & B. F. Barrentine. 1951. Use of calcium-45 in biological studies. Nucleonics. **8:** (3) 19–31.
4. Hevesy, G. 1948. Radioactive Indicators. Interscience Publ. New York, N. Y.
5. Kamen, M. D. 1951. Radioactive Tracers in Biology. Academic Press. New York, N.Y.
6. Siri, W. E. 1949. Isotopic Tracers and Nuclear Radiations. McGraw-Hill. New York, N. Y.
7. Whitehouse, W. J. & J. L. Putnam. 1953. Radioactive Isotopes. Clarendon Press. Oxford, England.
8. Nicolaysen, R., N. Eeg-Larsen & O. J. Malm. 1953. Physiology of calcium metabolism. Physiol. Revs. **33:** 424–444.
9. Laszlo, D., M. Blau, H. Spencer & J. Swernov. 1955. Biological studies on calcium, strontium, lanthanum and yttrium. Proc. Intern. Conf. Peaceful Uses Atomic Energy. A/Conf. 8/P/212.
10. Hansard, S. L., C. L. Comar & M. P. Plumlee. 1954. The effects of age upon calcium utilization and maintenance requirements in the bovine. J. Animal Sci. **13:** 25–36.
11. Hansard, S. L. & M. P. Plumlee. 1954. Effects of dietary calcium and phosphorus levels upon the physiological behavior of calcium and phosphorus in the rat. J. Nutrition. **54:** 17–31.
12. Kleiber, M., A. H. Smith, N. P. Ralston & A. L. Black. 1951. Radiophosphorus P^{32} as tracer for measuring endogenous phosphorus in cow's feces. J. Nutrition. **45:** 253–263.
13. Kleiber, M., A. L. Black, G. P. Lofgreen, J. R. Luick & A. H. Smith. 1955. Isotopes in research on animal nutrition and metabolism. Proc. Intern. Conf. Peaceful Uses Atomic Energy. A/Conf. 8/P/93.
14. Comar, C. L., R. A. Monroe, W. J. Visek & S. L. Hansard. 1953. Comparison of two isotope methods for determination of endogenous fecal calcium. J. Nutrition. **50:** 459–468.
15. Visek, W. J., R. A. Monroe, E. W. Swanson & C. L. Comar. 1953. Determination of endogenous fecal calcium in cattle by a simple isotope dilution method. J. Nutrition. **50:** 23–33.
16. Blau, M., H. Spencer, J. Swernov & D. Laszlo. 1954. Utilization and intestinal excretion of calcium in man. Science. **120:** 1029–1031.
17. Comar, C. L. & R. H. Wasserman. 1956. Radioisotopes in the study of mineral metabolism. *In* Progress in Nuclear Energy. **5:** Biol. Sci. Med. Pergamon Press. London, England. In press.
18. Wasserman, R. H., C. L. Comar & M. M. Nold. 1956. J. Nutrition. In press.
19. Plumlee, M. P. 1953. Placental transfer of calcium in cattle and rats using radioactive calcium-45. Ph.D. Thesis. Purdue Univ. Lafayette, Ind.
20. Lengemann, F. W., R. A. Monroe & C. L. Comar. 1955. In preparation.
21. Hansard, S. L., C. L. Comar & G. K. Davis. 1954. Effects of age upon physiological behavior of calcium in cattle. Am. J. Physiol. **177:** 383–389.
22. Thomas, R. O., T. A. Litovitz, M. I. Rubin & C. F. Geschickter. 1952. Dynamics of calcium metabolism. Time distribution of intravenously administered radiocalcium. Am. J. Physiol. **169:** 568–575.

23. Comar, C. L., W. E. Lotz & G. A. Boyd. 1952. Autoradiographic studies of calcium, phosphorus, and strontium distribution in the bones of the growing pig. Am. J. Anat. **90**: 113–129.
24. Visek, W. J., R. A. Monroe, E. W. Swanson & C. L. Comar. 1953. Calcium metabolism in dairy cows as studied with Ca^{45}. J. Dairy Sci. **36**: 373–384.
25. Swanson, E. W., R. A. Monore, D. B. Zilversmit, W. J. Visek & C. L. Comar. A study of variations in secretion of Ca^{45} by the mammary glands of dairy cows. In preparation.
26. Wilde, W. S., D. B. Cowie & L. B. Flexner. 1946. Permeability of the placenta of the guinea pig to inorganic phosphate and its relation to fetal growth. Am. J. Physiol. **147**: 360–369.
27. Semiannual Progress Report 1955. Univ. of Tennessee Agricultural Research Program-Atomic Energy Comm. ORO-145. Oak Ridge, Tenn.
28. Plumlee, M. P., S. L. Hansard, C. L. Comar & W. M. Beeson. 1952. Placental transfer and deposition of labeled calcium in the developing bovine fetus. Am. J. Physiol. **171**: 678–686.
29. Needham, J. 1931. Chemical Embryology. Cambridge Univ. Press. Cambridge, England.
30. Ellenberger, H. B., J. A. Newlander & C. H. Jones. 1950. Composition of the bodies of dairy cattle. Vermont Agr. Exptl. Stat. Bull. No. 558.
31. Pecher, C. & J. Pecher. 1941. Radioactive calcium and radioactive strontium metabolism in pregnant mice. Proc. Soc. Exptl. Biol. Med. **46**: 91–94.
32. Tyler, C. 1954. Studies on egg shells IV. J. Sci. Food Agr. **5**: 335–339.
33. Comar, C. L., I. B. Whitney & F. W. Lengemann. 1955. Comparative utilization of dietary Sr-90 and calcium by developing rat fetus and growing rat. Proc. Soc. Exptl. Biol. Med. **88**: 232–236.

CALCIUM AND PHOSPHORUS TRANSFER IN INTACT DAIRY COWS*

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It is fitting that one of the first papers in this monograph should deal with cows because, as regards lactation, the cow, in general, outranks woman. Before we talk of lactation, it is also fitting to talk of pregnancy, since pregnancy precedes lactation and gives lactation its biological significance. C. L. Comar concurred with this point of view, and suggested that he cover the subject of calcium metabolism in pregnancy and that we discuss the calcium metabolism involved in lactation, each of us presenting the work of both our laboratories relating to these respective subjects. We limited the scope of our papers accordingly.

The emphasis of this monograph is on calcium, but we started our work with tracers on lactation with phosphorus because P^{32} was available to us before Ca^{45}. In Oak Ridge, Tenn., Comar and his associates did excellent work on Ca transfer in lactation. Calcium and phosphorus metabolism are, however, so closely related that a joint report on the findings of our two laboratories is quite advantageous.

We shall discuss first the requirements of Ca and P for lactation, then the sources in blood of milk Ca and P. We shall show the evidence for the conclusion that a considerable part of the serine phosphate in casein is synthesized from inorganic phosphate in the udder. The relation of blood Ca and P to their source in the feed and in the skeleton will then lead to the final section dealing with the regulation of the calcium supply flow.

A high rate of lactation with a corresponding drain of Ca in some cases leads to "milk fever." We have started to analyze the biochemical conditions involved in milk fever. We are especially interested in the biochemistry of Boda and Cole's[1] prevention of milk fever by feeding low-calcium high-phosphorus diets to cows before they calved.

Calcium and Phosphorous Requirement for Lactation

The Ca and P content of milk is shown in TABLE 1.[2] TABLE 2 illustrates the problem with which a champion milk producer† (cow 346) is confronted. This daily requirement is a minimum for that level of lactation. Many, perhaps all, metabolic processes involve phosphate as an agent of energy transfer from catabolic to anabolic processes, from breakdown of fuel to the miraculous syntheses performed by living cells. Even transfer of simple substances such as glucose through membranes may involve phosphorylation, as Verzár and McDougall have suggested. We usually think of these phosphorylations as reversible or at least cyclical processes, in which the same phosphorus atom

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* The research of the "Davis Tracer Team" is supported by a contract of the University of California with the United States Atomic Energy Commission.

† California State Champion *Rutgers Posie Cutie*, winner of American Jersey Cattle Club AR Gold and Silver Medals. During one lactation she produced 891 lbs. butterfat (85 percent of her body weight) and 18,572 lbs. milk (18 times her body weight).

TABLE 1
CALCIUM AND PHOSPHORUS IN MILK

	Calcium	Phosphorus
Concentration, mg./100 ml. milk	125	108
per cent composition	*	†
(1) inorganic	100%	74%
(2) organic		
casein P		21%
lipoid P		3.7%
ribonucleic P		1.5%

* Calcium chloride, dicalcium phosphate.
† Mono magnesium phosphate, dicalcium phosphate, dipotassium phosphate.

TABLE 2
DAILY LOSS OF CALCIUM AND PHOSPHORUS IN MILK

	Cow 346	Cow 762
Milk production		
Total	34 kg./day	3.3 kg./day
Calcium	43 gm./day	4.2 gm./day
Phosphorus	33 gm./day	3.2 gm./day

performs the same function over and over again like the cog of a wheel in a machine. The cog of a wheel, however, wears down through use. Similarly, we expect some of the phosphorus atoms in the metabolic cycles to "get lost" as it were. We therefore also expect an increased requirement of P resulting from "wear and tear" whenever there is an increase in metabolic activity such as in milk production.

Blood as a Source of Calcium and Phosphorus in Milk

Utilization efficiency. The source of Ca and P in milk is the Ca and P in blood. TABLE 3 shows an average Ca and P content in a cow's blood and plasma. The distribution is based on an average of 12 determinations of

TABLE 3
CALCIUM AND PHOSPHORUS IN BLOOD AND PLASMA

	Calcium	Phosphorus	
		Total	Acid soluble
	mg.	*mg.*	*mg.*
(1) Per liter plasma	100	199	78
(2) Per liter blood*	62	197	81
(3) Per liter blood in plasma†	62	74	48
(4) Per liter blood in cells [(a)·1/161]	—	123	33
Per kg. body wt. in 45 ml. plasma	4.5	5.4	3.5
Per kg. body wt. in 72 ml. blood	4.5	14.2	5.8

* Almost none in cells.
† Note: $\frac{\text{Blood vol}}{\text{Plasma vol.}} = 1.61$ (mean of measurements on 12 cows at Davis, Calif.).

plasma per liter of blood, derived from centrifugation of blood samples, "hematocrit values."

Injections of labeled Ca and P salts into the bloodstream of lactating cows and goats indicate that Ca and P of milk can be derived from ionic forms of these elements in the bloodstream.

In 1935, Amaldi, D'Agostino, Fermi, Pontecorvo, Rasetti, and Segré[4] produced P^{32} by bombarding ordinary phosphorus with slow neutrons. Soon afterward, E. O. Lawrence obtained P^{32} by using CCl_4 as a target in the cyclotron. With a sample of this latter P^{32}, Aten and Hevesy,[5] in 1938, reported that they found radioactive P in casein prepared from the milk of a goat within 2 hours after subcutaneously injecting P^{32} as sodium phosphate.

The first tracer experiment on Ca transfer from an inorganic form in blood to milk was performed by Erf and Pecher.[6] They injected Sr^{89} as a tracer for Ca into the bloodstream of 2 cows and measured the appearance of the radioactive strontium in the milk.

FIGURE 1 shows the results of this experiment, together with similar findings on phosphorous transfer to milk that were published 8 years later by the Davis Tracer Team.[7] The maximum labeling for P^{32} is reached at approximately the same time as that for Sr^{89}, namely, about 5 hours after injection.

The difference in the labeling of milk by Sr and P may indicate either a relatively larger pool of mobile P than of mobile Ca or a greater mobility (measured as turnover rate) of the calcium pool, provided that Sr is really a valid tracer for Ca.

On the average, a greater part of the injected Ca^{45} than of the injected P^{32}

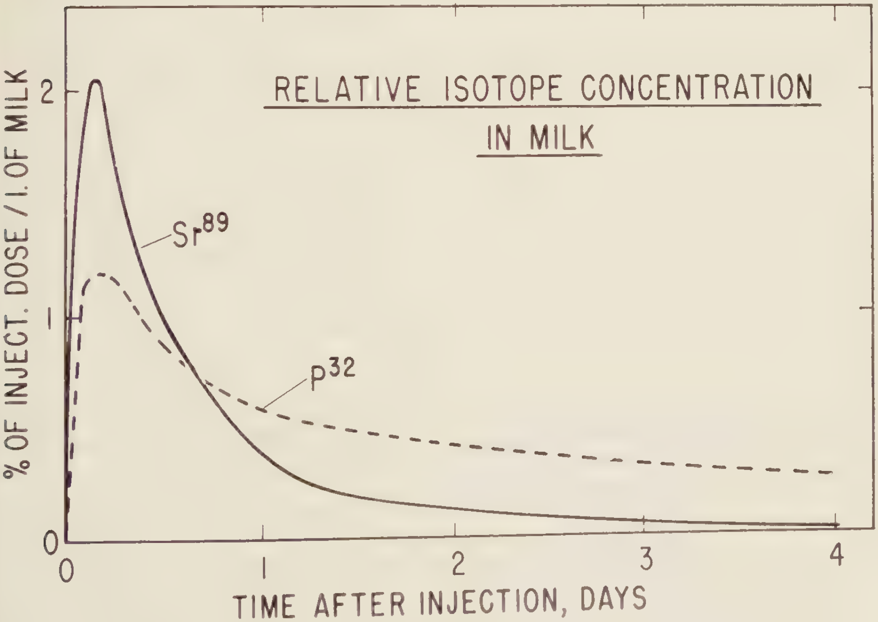

FIGURE 1.

TABLE 4

TRANSFER OF INTRAVENOUSLY INJECTED Ca^{45} AND P^{32} TO MILK IN 7 DAYS

Trial	Milk yield in 7 days		per cent of injected dose in milk	
	Ca trial kg.	P trial kg.	Ca^{45} %	P^{32} %
(1)	59	66	35.8	20.6
(2)	53	59	33.7	23.7

Ratio of per cent transfer (mean) Ca:P = 1.6:1

Ca^{45} trials: Visek *et al.*[8]
P^{32} trials: Kleiber *et al.*[7]

appears in the milk. This conclusion follows from a comparison of early work of the Davis Tracer Team[7] with those of Visek *et al.*[8] (FIGURE 4)

Transfer quotient. One may ask how much of the Ca and P in the milk (or, for P, the casein in particular) comes from the inorganic Ca and P in the blood. We have called the answer to that question the "transfer quotient". If in a time-element, dt, the specific activity in the plasma precursor is π, and if the rate of transfer of Ca or P from that precursor to the product is p, then the amount of tracer transferred to that product in the time interval dt is $p\pi dt$. The amount transferred in the time interval $t_2 - t_1$ is consequently $p \int_{t_1}^{t_2} \pi dt$. This amount of tracer is also the amount present in the milk collected between $time_1$ and $time_2$ provided that no delay is involved in the transfer. This amount present in the milk is the specific activity in the milk at time t_2, λ_2, times the amount of milk Ca (or P). The latter is the product of rate of production, l, and the time interval, $t_2 - t_1$. Thus the amount of tracer in the milk is $l(t_2 - t_1)\lambda_2$. If no delay is involved, the amount of tracer collected in the milk and the amount transferred from the original precursor must be equal. Therefore,

$$p \int_{t_1}^{t_2} \pi dt = l(t_2 - t_1)\lambda_2 ,$$

and the ratio of the rate at which the precursor contributes to the formation of the product, and the rate at which the product is formed, is

$$q = \frac{p}{l} = \frac{\lambda_2}{\int_{t_1}^{t_2} \pi dt} (t_2 - t_1).$$

By 2 daily intravenous injections of the tracer into a cow over a 20-day period we could establish quasi-constant levels of specific activity of P^{32} in plasma and milk during the second half of the period. For a case such as this the equation for the transfer quotient is simplified to

$$q = \frac{p}{l} = \frac{\bar{\lambda}}{\bar{\pi}}$$

where $\bar{\lambda}$ and $\bar{\pi}$ are constant specific activities.

FIGURE 2 shows the levels of specific activity. We were surprised to find

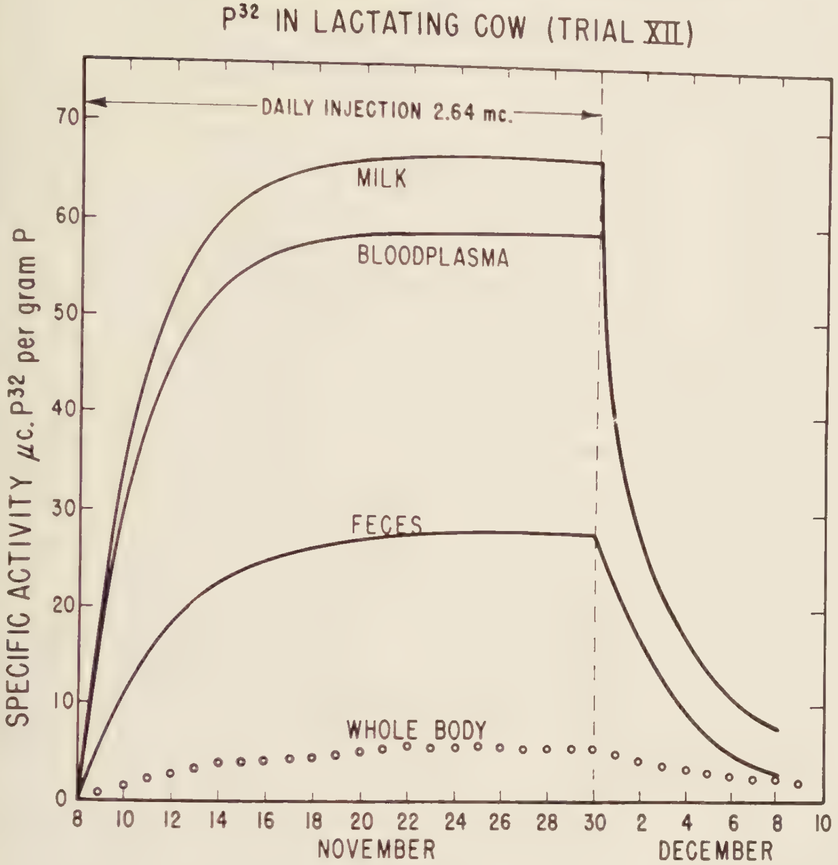

FIGURE 2.

a consistently higher specific activity in the milk than in the blood. How could more than all of the milk phosphorus come from the inorganic P in the blood?

A possible explanation of the result is the timing of our injections and milkings. The rate of milk production apparently is not constant as we have assumed. It seems to be greater when the udder is empty, and to decrease as the hydrostatic pressure in the udder increases.[9]

A greater proportion of the milk was therefore produced following the injection when the P^{32} activity of the blood was higher, and less was produced when the activity of the blood was low. This effect may explain our results.

Visek *et al.*,[8] however, observed a higher specific activity in milk than in blood, even several milkings after a single injection of the tracer.

FIGURE 3 illustrates this observation. A delay in transfer might explain the result, but this delay would have to be greater than that usually found. Furthermore, FIGURE 4 indicates that the time integrals of specific activity in milk is greater than that in blood.

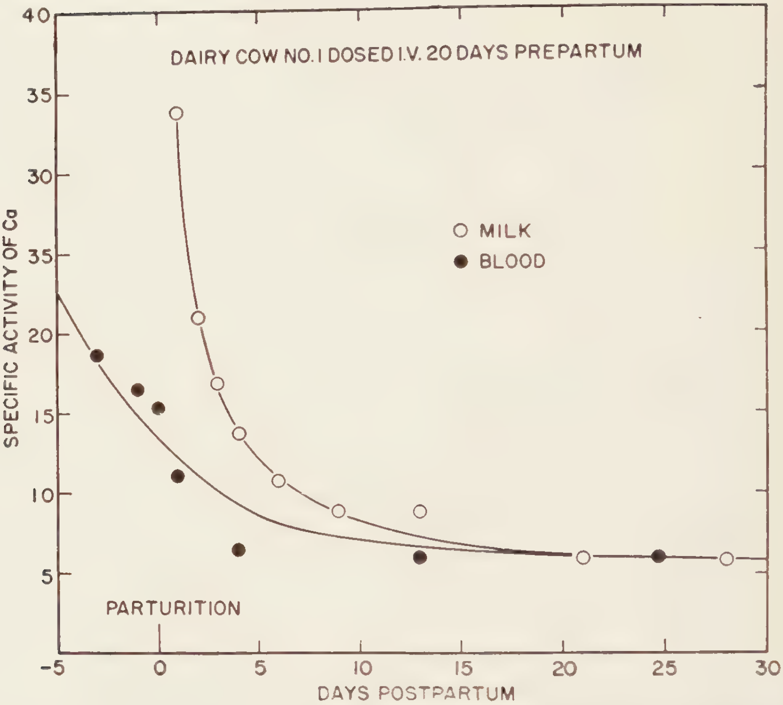

FIGURE 3. The specific activities of the calcium in blood and milk plotted as a function of time after intravenous injection of Ca^{45} at 20 days prepartal—Visek *et al.*[8]

Saarinen *et al.*[10] administered to a cow, orally, 200 ml. of a solution with P^{32} as disodium phosphate. They confirmed the result of Aten and Hevesy mentioned above that the milk P is not derived from plasma phospholipid, but from inorganic plasma P. They also noted that the specific activity of milk P was always higher than that of the plasma acid-soluble P. They concluded that "possibly only a fraction of the phosphates usually determined as blood plasma inorganic phosphates serves as the main precursor of the phosphorus in milk."

At present this seems to be the most probable explanation for the paradox of a higher specific activity in the product than in the precursor. To equate acid soluble P in blood with inorganic P in blood may be an error. Trichloroacetic acid may liberate P from organic bonds that, in blood, prevent a rapid exchange with ionic phosphorus.

In some cases a collecting pool may lead to a product with a higher specific activity than that observed in the precursor at a given time. For calcium in colostrum, Visek *et al.*[8] write, "These results indicate that much of the radiocalcium found in colostrum probably entered the mammary gland at a time when the specific activity of the blood calcium was high and remained there until the animal was milked."

FIGURE 5, obtained with our analog computer, illustrates, for a 4-pool system

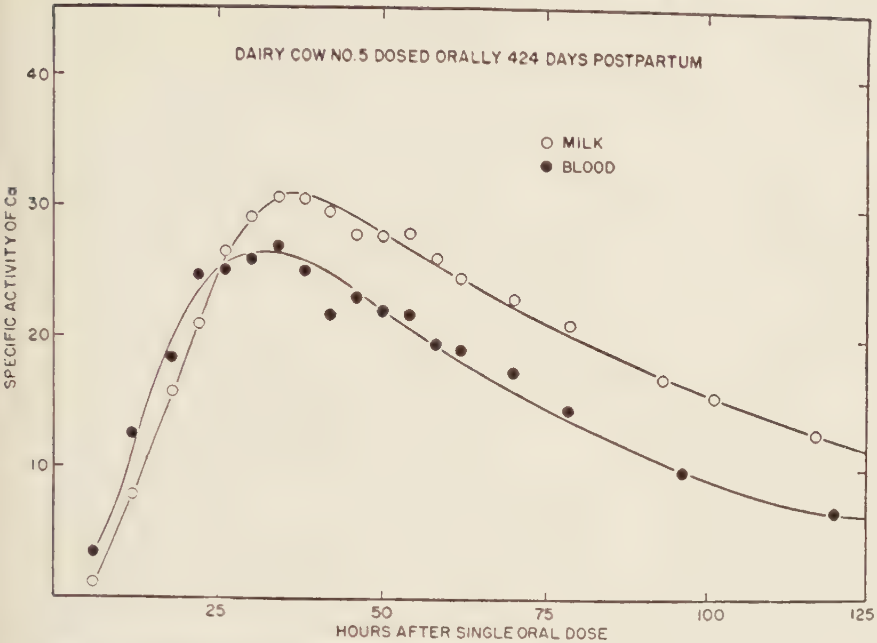

FIGURE 4. The specific activities of the calcium in blood and milk plotted as a function of time after oral administration of Ca^{45}—Visek *et al.*[8]

with mixing and dilution, the higher specific activity in a collecting pool (ϵ tot) over that in the donor pool (γ).

Place of Utilization of Inorganic P in Milk Production

The results of the Aten and Hevesy investigations indicate that the utilization of inorganic P for casein synthesis occurs in the udder. The possibility of an unidentified organic precursor formed in the liver exists, however, and with the usual procedure such a compound might be measured together with the inorganic blood phosphorus. Amputation of the udder would certainly eliminate the liver, and might show that the udder *can* utilize inorganic P for casein synthesis. Since, however, amputation produces unphysiological conditions, and since we specialize in work with intact cows, we have used another method that proves just as conclusively as measurements with amputated udders or, in fact, more so, that at least a considerable part of the utilization of inorganic P does occur in the udder itself.

Our method is to inject the labeled phosphate into one cystern of a normal lactating cow. If all the casein P were derived from inorganic P via a process outside the udder, the casein P from all quarters should have the same specific P^{32} activity.

FIGURE 6 shows the first milk samples, 3 hours after injection of the P^{32}. The casein from the injected quarter has a specific activity 11 times that of the specific activity in the casein from the 3 noninjected quarters. Even 2½ days after the injection the P of the casein from the injected quarter is twice as radioactive as that from the other quarters. This observation proves that utilization of inorganic P for casein synthesis occurs in the udder.

Source of P and Ca in Blood

Utilization of Ca and P in feed. The National Research Council, Washington, D.C., recommends 1 gm. Ca and 0.7 gm. P in the feed per pound of milk produced.[11] To make use of these recommendations, we are interested in the digestibility of Ca and P in feeds.

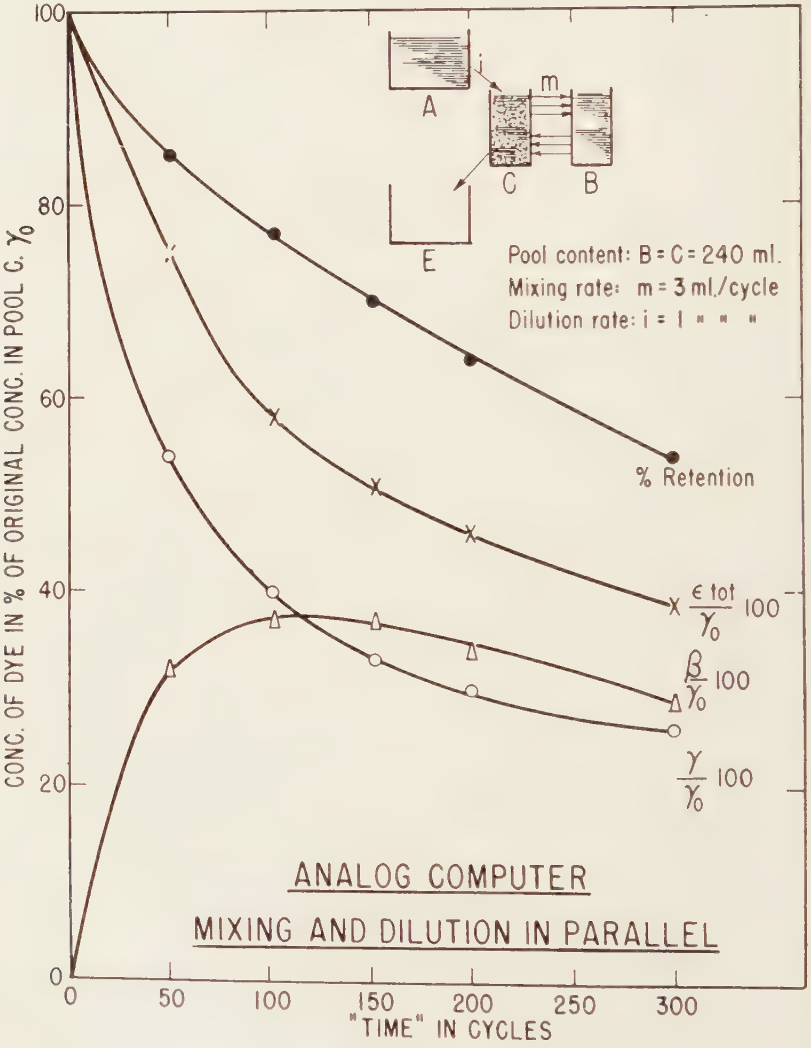

FIGURE 5.

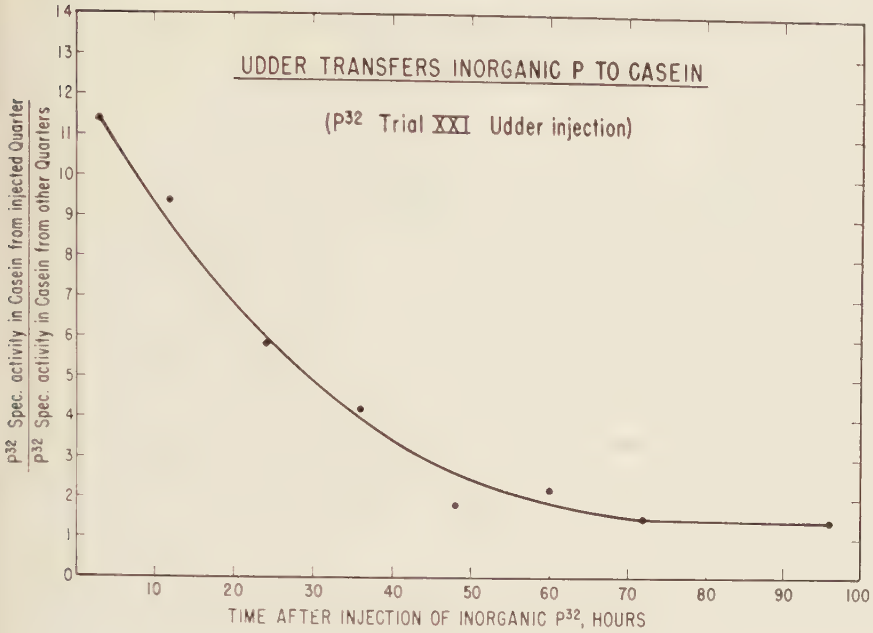

FIGURE 6.

A considerable part of fecal P comes from the blood.* We have determined the ratio of endogenous to exogenous P in feces of cows by a method of dynamic isotope dilution.[13]

By 2 intravenous injections per day, an almost constant level of specific activity in plasma and in feces can be attained in about 10 days. Such quasi-constant levels can be maintained for another 10-day period. The ratio of these 2 levels indicates the part of the fecal P that came to the feces by secretion from the body, and is known as endogenous fecal P. The calculation is explained by FIGURE 7.

A stream of undigested feed P joins a stream of endogenous P. If the rate of flow of the latter is s and the specific activity of P^{32} in it is π, then the rate of flow of the tracer in this stream is $s\pi$. If the rate of flow of total fecal phosphorus is f, and if the specific activity of fecal P is ϕ, then the rate at which P^{32} is excreted in feces is $f\phi$. Since the P^{32} in feces can only come from the blood, the rate of fecal loss is equal to the rate of secretion of endogenous P into the intestinal content. That is,

$$f\phi = s\pi,$$

or

$$q = \frac{s}{f} = \frac{\phi}{\pi}; \qquad s = f\frac{\phi}{\pi}.$$

---

* The first measurement of endogenous fecal phosphorus was carried out by Hevesy, Hahn, and Rebbe in man in 1939. They used the specific activity of the urinary P as an indication of endogenous part of fecal P. See G. Hevesy.[12]

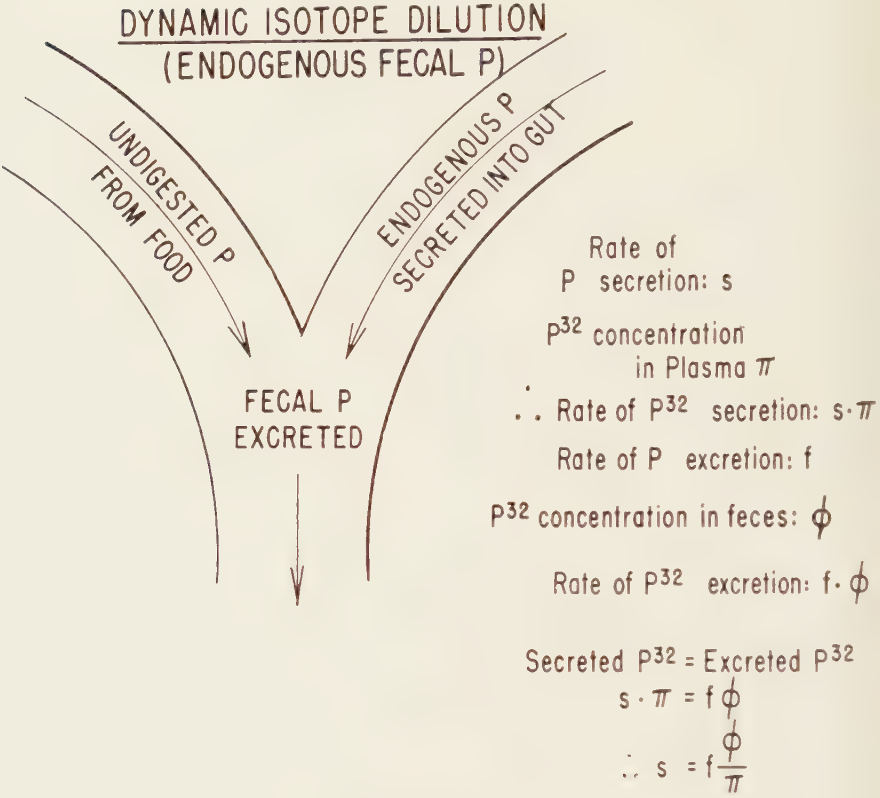
DYNAMIC ISOTOPE DILUTION
(ENDOGENOUS FECAL P)
UNDIGESTED P FROM FOOD
ENDOGENOUS P SECRETED INTO GUT
FECAL P EXCRETED
Rate of P secretion: s
P^{32} concentration in Plasma π
$\therefore$ Rate of P^{32} secretion: $s \cdot \pi$
Rate of P excretion: f
P^{32} concentration in feces: ϕ
Rate of P^{32} excretion: $f \cdot \phi$
Secreted P^{32} = Excreted P^{32}
$s \cdot \pi = f \phi$
$\therefore s = f \frac{\phi}{\pi}$

Figure 7.

Table 5

Variability of "True" Digestibility and Ca-Requirement
Two cows on same feed.

Cows	VI		VII	
"True" digestibility of Ca		17%		56%
Ca in feed per day, gm. . .		28.0		29.0
Ca absorbed per day, gm.		4.8		16.2
Ca secreted per day to:				
Feces, gm. . . .	6.7		7.2	
Urine, gm.	0.8		0.5	
Milk, gm. . .	6.3		9.9	
Total Ca secret./day, gm.		13.8		17.6
Ca req. from stores/day, gm. . .		9.0		1.4
(Fecal Ca per day, gm. . . .		30.0		20.0)

If i is the rate of P intake in food, then $\frac{i-f}{i}$ is the apparent digestibility and $\frac{i-(f-s)}{i} = 1 - \frac{f-s}{i} = 1 - \frac{f}{i}\left(1 - \frac{\phi}{\pi}\right)$ is the "true" digestibility, which is also $\frac{a}{i}$, where a is the rate of absorption of feed P. In one trial in which the apparent digestibility of P was only 11 per cent, the "true" digestibility was 50 per cent.

Visek *et al.*[14] have applied the same method to the determination of the true digestibility of Ca. TABLE 5 shows the result of their determination, and demonstrates the great variability in the "true" digestibility of calcium from the same ration fed to 2 cows. The table shows that, of 2 cows, the one with

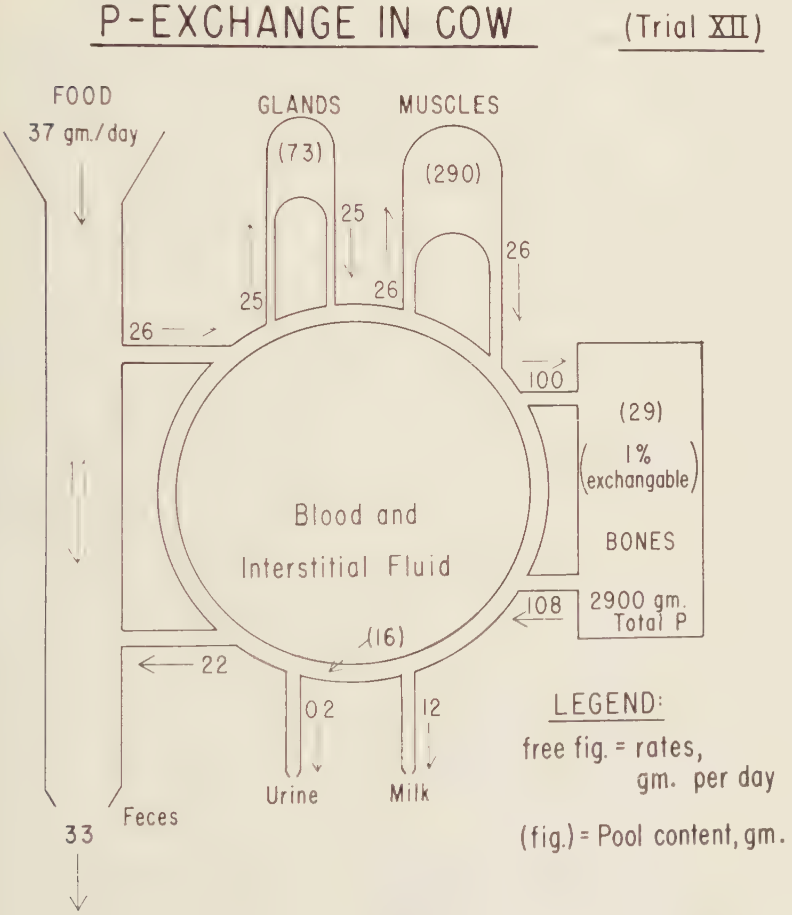

FIGURE 8.

the lower rate of milk production but fed the same ration, lacked 9.0 gm. of Ca per day. The other lacked only 1.4 gm.

Ca from bones as stores mobilized for lactation. The shortage of calcium intake from the feed, 9.0 gm. per day in one cow, 1.4 gm. per day in the other, has to be supplied from the skeleton, the main Ca store of the body. In times of great need for lactation, the tapping of the skeleton as a store of Ca seems to be a normal periodic occurrence. It has been described by Goss and Schmidt[15] for lactating rats fed a low-calcium diet. On low Ca intake, female rats, in 2 lactations, removed 70 per cent of their skeletal Ca and secreted it in milk. If the cow described above needed 9 gm. Ca per day, she would have depleted her labile Ca pool of 2 per cent of her bone calcium, or approximately 160 gm.[16] in 18 days. It is obvious that the cow must tap a greater Ca pool during high lactation.

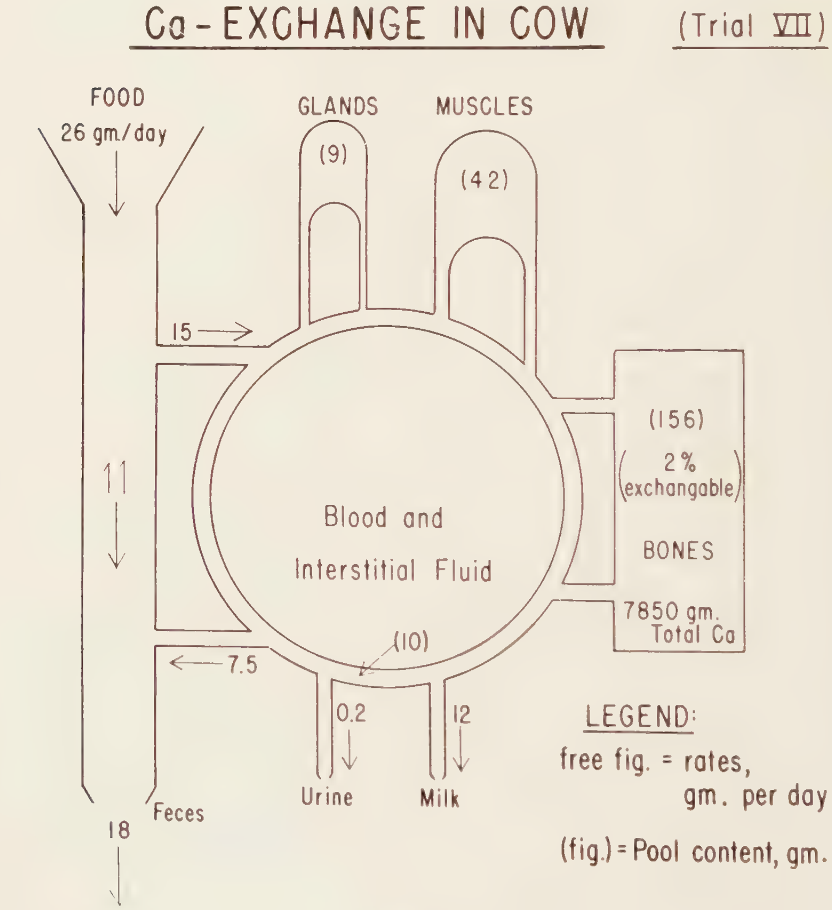

Figure 9.

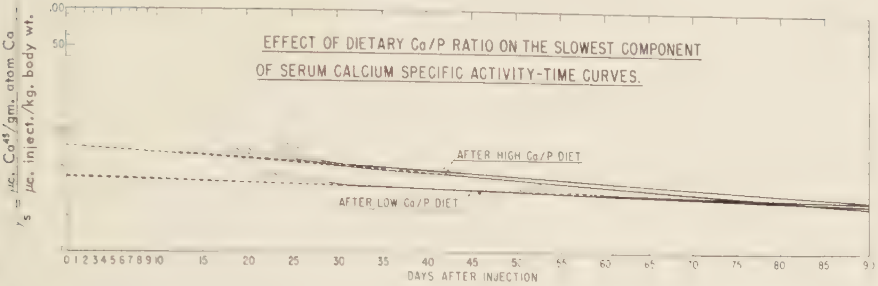

FIGURE 10.

Regulation of Ca and P Supply

From measurements of turnover rates of various P pools and estimates of the contents of these pools from anatomical data, the P metabolism of a cow was represented in a scheme (FIGURE 8).

The labile P pool in the skeleton, 1 per cent of the P in the bones, was estimated by Black *et al.*,[17] based on the assumption that the exchange between inorganic P in blood and labile bone P is a rapid process.

From measurements of Hansard *et al.*[16] and J. Luick[15] we can summarize the Ca content and transfer rates in FIGURE 9.

Where is the regulator for Ca supply most active? The great variability of "true" digestibility suggests that this might be a function that could be affected to a great extent by a regulator. The rate of secretion of endogenous Ca could also be expected as a suitable point of influence by a regulator. At the present state of our work, mobilization of bone Ca seems to be a particularly important function of the regulator.

J. Luick noted that the ratio of Ca/P in the diet, which presumably affects the activity of the parathyroid system (Boda and Cole[1]), did not greatly affect the turnover rate of Ca pools, but did have a decisive influence on the size of the pool. This pool size is estimated, after injection of radio calcium, by extrapolating the logarithm of specific activity in Ca of serum to time of injection. The extrapolated specific activity, multiplied by the pool content, should be equal to the injected dose provided that the pool of available P includes the smaller pools with greater turnover rates. The pool content, therefore, is the injected dose divided by the specific activity extrapolated to time of injection.

FIGURE 10 shows the result of the work of J. Luick and indicates that feeding a cow with a low Ca-high P ration before calving tends to increase the size of the available Ca pool, and to decrease rather than increase the turnover rate of this pool.

Conclusions

(1) A dairy cow in high production may give off daily in her milk 43 gm. of calcium and 37 gm. of phosphorus.

(2) Intravenous injection of P^{32} as a tracer indicated that most of the P in

milk, even of the P in the serine phosphate of the casein, originates from the acid-soluble P in the blood plasma.

(3) Injection of P^{32} into the cystern of one quarter of the udder proves that a considerable part of the utilization of inorganic P for casein synthesis occurs in the udder.

(4) After intravenous injection of Ca^{45} and of P^{32} into cows producing from 53 to 66 kg. of milk in 7 days, 35 per cent of the injected Ca^{45} and 22 per cent of the injected P^{32} appeared in the milk.

(5) After intravenous injection of Ca^{45} and of P^{32}, the specific activity of the tracer was considerably higher in the milk than in the blood plasma. For phosphorus, the most likely explanation of this observation at present is that by Saarinen *et al.*,[10] who suggested that only a fraction of phosphates determined as plasma in organic phosphate serves as the main precursor of milk phosphorus.

(6) Intravenous injection of P^{32} or of Ca^{45} makes possible the determination of the P or Ca that comes to the feces from the blood (endogenous fecal P or Ca).

(7) The "true" digestibility of Ca of 2 cows on the same feed varied greatly. The requirement of a Ca supplement from the skeletal Ca store therefore differed considerably.

(8) The regulation of P and Ca supply is explained by schemes of the P and Ca pools, and transfer rates of lactating cows.

(9) Feeding a low Ca-high P ration increased the amount of skeletal Ca available for mobilization and decreased the turnover rate of this pool of available Ca.

References

1. Boda, J. M. & H. H. Cole. 1954. The influence of dietary Ca and P on the incidence of milk fever in dairy cattle. J. Dairy Sci. **37:** 360–372.
2. Macy, I. G., H. J. Kelly & R. E. Sloan. 1953. The Composition of Milks. National Research Council. Publication 254. Washington, D. C.
3. Verzár, F. & E. J. McDougall. 1936. Absorption from the intestine. Longmans, Green. London, England.
4. Kamen, M. 1947. Radioactive Tracers in Biology. : 184. Academic Press. New York, N. Y. [See text, page 301, reference[4], Amaldi, D'Agostino, Fermi, Pontecorvo, Rasetti, and Segré]
5. Aten, A. H. W. & G. Hevesy. 1938. Formation of milk. Nature. **142:** 111.
6. Erf, L. A. & C. H. Pecher. 1940. Secretion of radio-strontium in milk of two cows following intravenous administration. Proc. Soc. Exptl. Biol. Med. **45:** 762.
7. Kleiber, M., A. H. Smith & N. P. Ralston. 1948. Secretion in cow's milk of intravenously injected radioactive phosphorus P^{32}. Proc. Soc. Exptl. Biol. Med. **69:** 354–356.
8. Visek, W. J., R. A. Monroe, E. W. Swanson & C. L. Comar. 1953. Calcium metabolism in dairy cows as studied with Ca^{45}. J. Dairy Sci. **36:** 376.
9. Petersen, W. E. 1939. Dairy Science. : 313. Lippincott. New York, N. Y.
10. Saarinen, P., C. L. Comar, S. P. Marshall & G. K. Davis. 1950. Partition of orally administered radioactive phosphorus in the blood and milk of the dairy cow. J. Dairy Sci. **33:** 878–884.
11. Maynard, L. A. 1947. Animal Nutrition. : 439. 2nd ed. McGraw-Hill. New York, N. Y.
12. Hevesy, G. 1948. Radioactive Indicators. : 103. Interscience. New York, N. Y.
13. Kleiber, M., A. H. Smith, N. P. Ralston & A. L. Black. 1951. Radiophosphorus as a tracer for measuring endogenous phosphorus in cow's feces. J. Nutrition. **45:** 253–263.
14. Visek, W. J., R. A. Monroe, E. W. Swanson & C. L. Comar. 1953. Determination

of endogenous fecal calcium in cattle by a simple isotope dilution method. J. Nutrition. **50:** 23–33.

15. Goss, H. & C. L. A. Schmidt. 1930. Calcium and phosphorus metabolism in rats during pregnancy and lactation and the influence of the reaction of the diet thereon. J. Biol. Chem. **86:** 417–432.
16. Hansard, S. L., C. L. Comar & G. K. Davis. 1954. Effect of age upon the physiological behavior of calcium in cattle. Am. J. Physiol. **177:** 383–389.
17. Black, A. L., M. Kleiber, A. H. Smith & N. P. Ralston. 1953. Mobility of skeletal P in a mature dairy cow as determined with radioactive phosphorus. Proc. Soc. Exptl. Biol. Med. **82:** 248–252.
18. Luick, J. 1956. Ph.D. thesis. Nutrition. Univ. of California.

ABSORPTION AND METABOLISM OF CALCIUM IN HUMAN BEINGS, STUDIED WITH CALCIUM^{45} *

By Felix Bronner† and Robert S. Harris
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The use of the metabolic balance technique in the study of calcium metabolism has led to the development of several classical concepts of mineral metabolism. Net *calcium retention* is one example. Yet until it became possible to use isotopes in studies of metabolism, the related concept of *calcium absorption* was not well defined, because it was impossible to differentiate endogenous from exogenous calcium in the feces. The availability of radiocalcium (Ca^{45}) for use in studies with human beings has provided a stimulus to reinvestigate some of these problems of calcium metabolism.

"Boys aged 11 to 16 years, and two adults aged 21 and 22 years served as subjects. Though all were institutionalized for mental inadequacy, the boys were otherwise normal and in good health; the two adults had spastic paralysis, but neither clinical nor laboratory examination revealed any abnormalities in their calcium metabolism. All subjects were prepared by receiving daily one quart of milk and one multivitamin tablet‡ as a supplement to their institutional diet, beginning at least two weeks before the study and continuing until the end of the program. The diet was found by analysis to approximate the Recommended Dietary Allowance of the National Research Council for males of these ages.[1] The subjects abstained from food for 12 hours prior to each experiment, then received one of the standard test breakfasts. These consisted of either 180 gm. cooked oatmeal (approximately 35 gm. rolled oats, 8.3 per cent moisture) and milk, or 240 gm. cooked farina (approximately 30 gm. farina, 10.5 per cent moisture) and milk. In the moderate-calcium breakfasts 240 ml. milk were given. In the low-calcium studies, only 60 ml. milk were given, and the solids contents of the breakfast was made up approximately by additional farina (about 26 gm. dry basis). The Ca^{45} was given in 60 ml. milk which were intimately mixed with the cereal, while the remainder of the milk was drunk. When the isotope was given intravenously, it was injected 30 minutes after the breakfast. Serum, urine, and feces samples were analyzed for total calcium by a modification of the method of Salomon, Gabrio, and Smith,[2] and for Ca^{45} by counting calcium oxalate samples which contained 4.0 mg. calcium."[3]

The skeleton undoubtedly plays a dominant role in calcium metabolism, since its concentration of calcium far exceeds that of any other calcium compartment (FIGURE 1). Though possibly only 0.2 per cent[4] and probably no more than 2 per cent[5] of the calcium in the skeleton is readily available for exchange, this still represents 10 to 100 times the quantity of calcium present

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‡ Vi-Penta Perles, generously donated by Hoffman-LaRoche, Inc., Nutley, N. J.

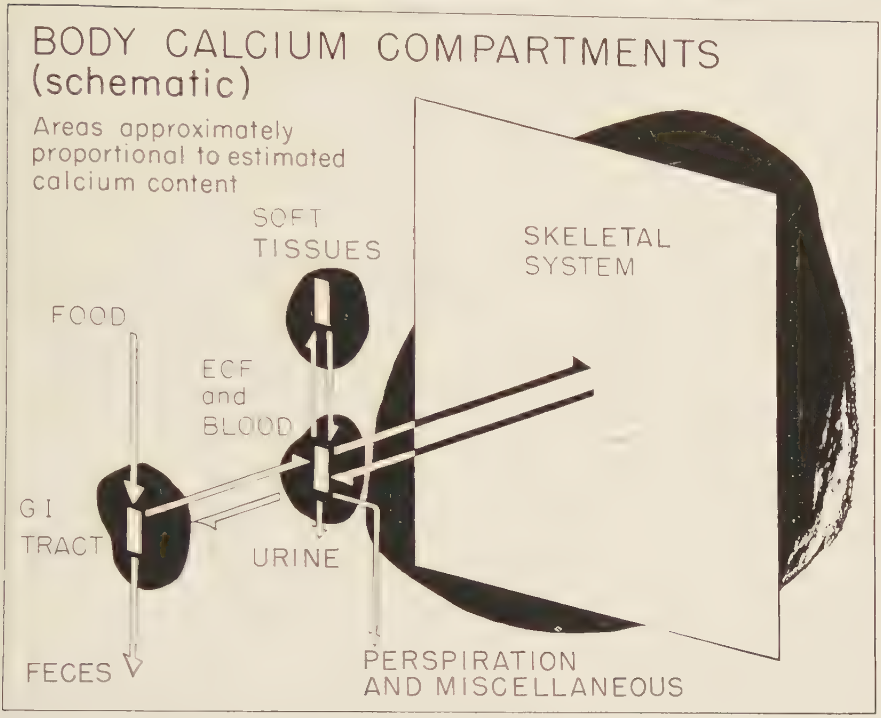
BODY CALCIUM COMPARTMENTS
(schematic)
Areas approximately proportional to estimated calcium content
SOFT TISSUES
SKELETAL SYSTEM
FOOD
ECF and BLOOD
G I TRACT
URINE
FECES
PERSPIRATION AND MISCELLANEOUS

FIGURE 1.

in the serum. It is not surprising, therefore, that more than four fifths of the injected calcium had disappeared from the circulation within 5 minutes (FIGURE 2), and that the specific activity of calcium in the serum had not leveled off even 2 months after injection.

It seemed of interest to derive equations that would represent analytically the course of disappearance of injected Ca^{45} (FIGURE 2). In the case of the boys, the equation was derived by an iteration procedure applied to the best visual curve drawn through the experimental points,[6] while that of the older individual was derived graphically (see Flexner *et al.*[7] for a detailed description of the method). Both equations were derived for a common body weight of 35.6 kg. The half-time values listed in TABLE 1 indicate that an appreciable difference existed between the group of boys and the older individual. With the exception of the very uncertain first half-time value, it is seen that the injected Ca^{45} disappeared from the blood of the boys 5 to 10 times faster than from the blood of the young adult. Until the size of the various calcium compartments is analyzed independently, these equations cannot reveal whether the adults differed from the group of boys by virtue of differences in the size of calcium compartments, differences in equilibration rates, or both. The data of urinary and fecal output[6] suggest a smaller retention on the part of the older person. While 1 individual is not necessarily representative of a group, it is noteworthy that the course of disappearance of absorbed calcium

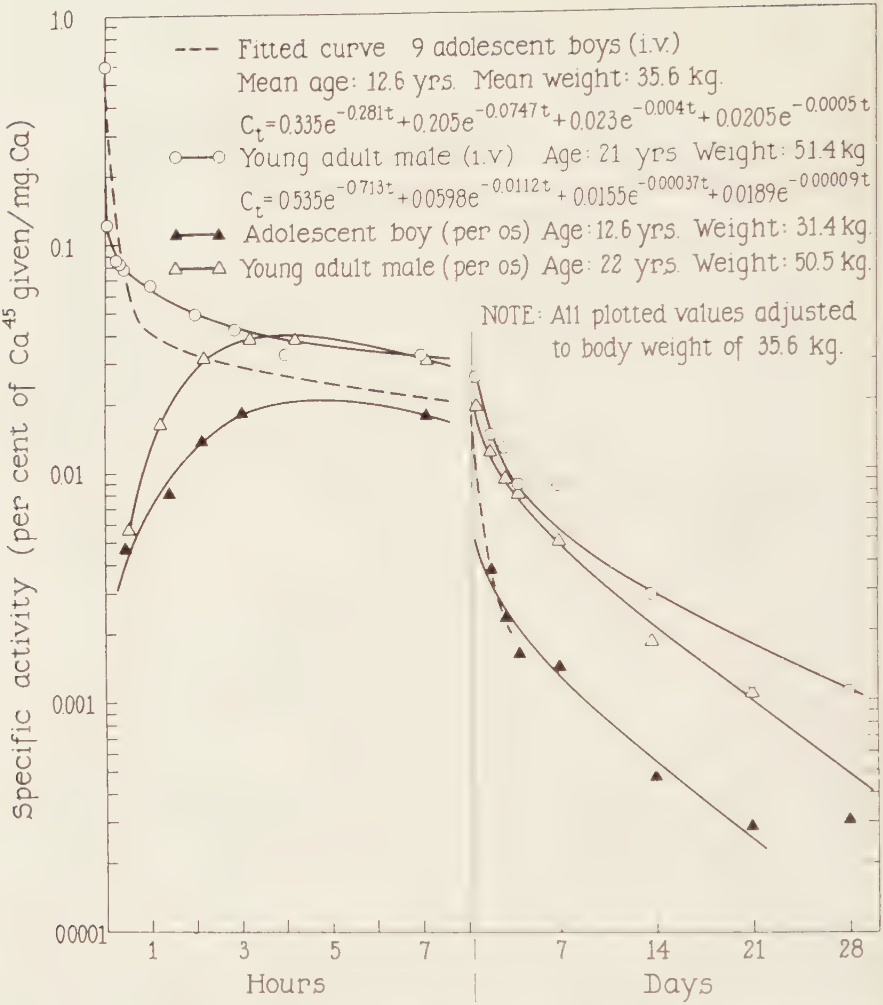

FIGURE 2. Disappearance of injected and ingested Ca^{45} from human serum; 0.7 and 2 microcuries were injected into boys and adult, respectively. The boys and adult ingested 4.1 and 4.4 microcuries, respectively.

TABLE 1

HALF-TIME VALUES, DISAPPEARANCE EQUATIONS

Boys		Adult	
Term	$T_{\frac{1}{2}}$	Term	$T_{\frac{1}{2}}$
$0.335e^{-0.281t}$	2.5 min.	$0.535e^{-0.713t}$	1.0 min.
$0.205e^{-0.075t}$	9.3 min.	$0.0598e^{-0.0112t}$	62 min.
$0.0234e^{-.004t}$	173 min. (2.9 hrs.)	$0.0155e^{-0.0004t}$	1850 min. (30.9 hrs.)
$0.0205e^{-.00005t}$	1350 min. (22.6 hrs.)	$0.0189e^{-0.00009t}$	7560 min. (12.6 hrs.)

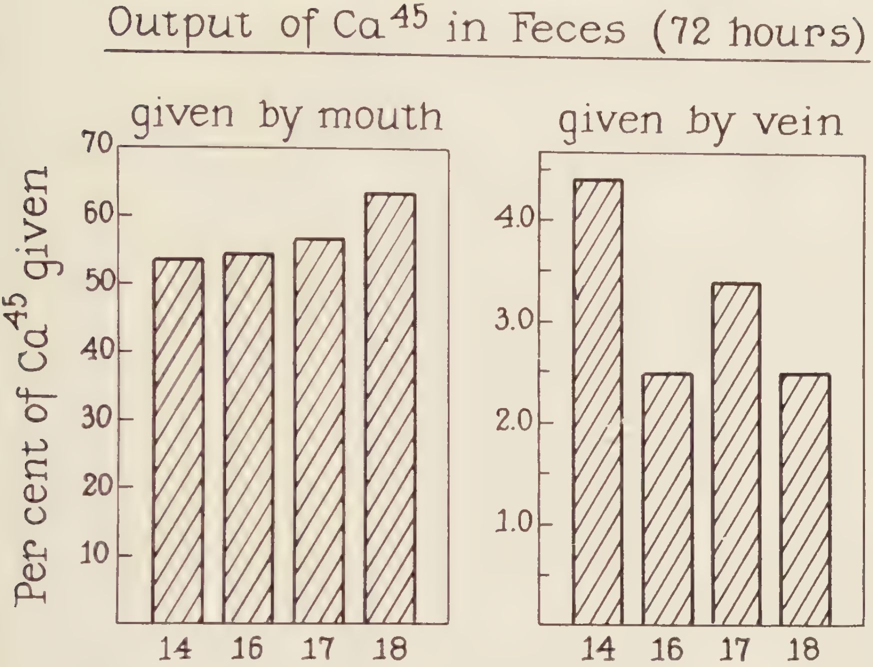

FIGURE 3. Output of Ca^{45} in feces of adolescent boys in 2 successive experiments. Collection period: 72 hours. Test breakfast consisted of 35 gm. rolled oats (8.3 per cent moisture) and 240 ml. milk. Isotope injected 30 minutes after the test breakfast, or diluted in 60 ml. milk intimately mixed with the breakfast cereal. Calcium intake: 294 mg. in ingestion experiment, 329 mg. in injection experiment. Ca^{45} output for hours 72 to 120 did not exceed 5 per cent of dose for any individual in a companion ingestion experiment.

from the serum of the 1 adult closely paralleled that of the other who had received the isotope by vein (FIGURE 2).

Schematization of calcium metabolism would suggest a series of rate processes that involve absorption, transport, bone salt formation, bone salt resorption, and excretion. We shall now discuss some evidence that suggests that neither calcium absorption, calcium excretion, nor calcium transport are likely to be the rate-limiting steps in such a chain of events.*

Absorption

FIGURE 3 shows that under the conditions of the standard breakfast nearly half of the Ca^{45} eaten was recovered from the feces in 3 days, yet during a like period the same individual lost in the feces less than 5 per cent of the Ca^{45} he had received by intravenous injection. This suggests that only an inappreciable fraction of absorbed calcium is re-excreted in the feces within 72 hours, and that the Ca^{45} recovered from a single test meal during such a period largely represents unabsorbed calcium.

FIGURE 4 shows that two groups of 10 and 9 boys, respectively, who were on the same general institutional diet and who were given similar test break-

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* This discussion assumes that calcium metabolism is normal and that intake is sufficient to meet the body's needs. We have not studied the effect of disease on these processes or their interrelationships.

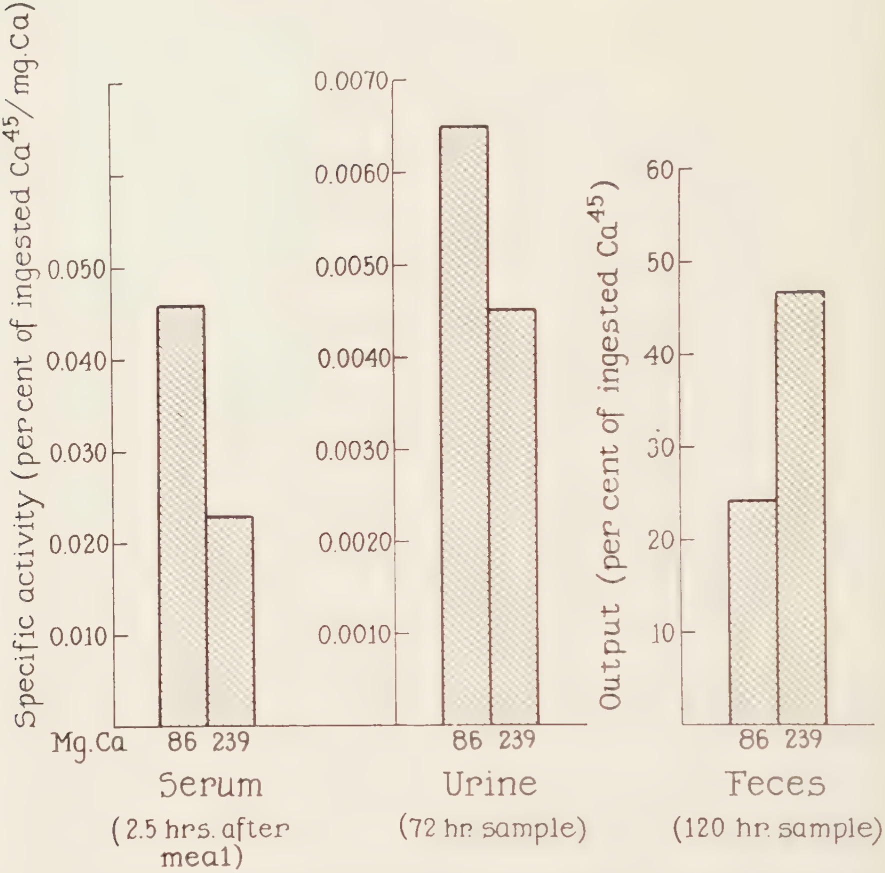

FIGURE 4. Effect of 2 levels of calcium intake on the disposition of ingested Ca^{45} by 2 groups of 10 and 9 adolescent boys, respectively. Test breakfast consisted of 30 gm. farina (12.5 per cent moisture) and of either 60 or 240 ml. milk. Note agreement in fecal output data of this with the experiment shown in FIGURE 3.

fasts, excreted proportionately more calcium when their calcium intake increased. On the basis of the data in FIGURES 3 and 4, one is led to conclude that proportionately less calcium is absorbed as calcium intake increases

The graph in FIGURE 5 illustrates the same point. Subject No. 55, a 13-year-old boy, passed in his stools in 5 days 45 per cent of the Ca^{45} in his test meal while subject No. 56, a 22-year-old adult, whose meal contained only one third as much calcium as that of the boy, excreted in his feces only 17 per cent of the ingested Ca^{45} during a comparable span of time. Since the adult had excreted nearly 5 times as much Ca^{45} in his urine in 5 days as the boy, it is probable that the need for calcium (as reflected by calcium retention) does not appreciably affect absorption, but that intake does.

These observations suggest that, over the range studied, the per cent of calcium absorbed decreased as intake increased. It is likely that the per cent of calcium absorbed is an exponential function of the calcium intake,[8] and that

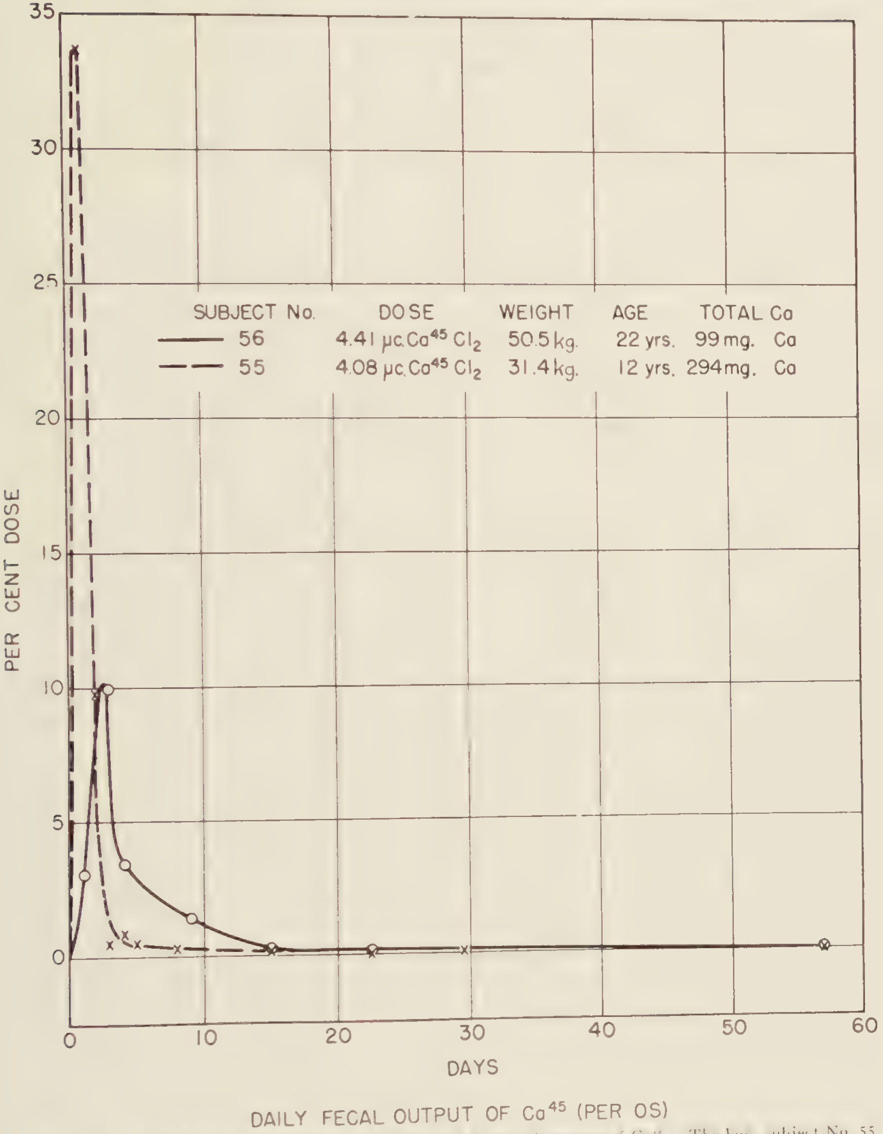

FIGURE 5. Output of Ca^{45} in the feces of 2 subjects following ingestion of Ca^{45}. The boy, subject No. 55, ingested 57 gm. farina (10.5 per cent moisture) and 240 ml. milk, while the adult, subject No. 56, ingested 105 gm. farina (10.5 per cent moisture) and 60 ml. milk. Ca:P ratio 1.0 and 0.6, respectively. Blood disappearance curves for these subjects are shown in FIGURE 2.

absorption becomes insensitive to intake when the body's needs for calcium have been met.

Excretion

Crawford and his collaborators[9] have recently reported that the renal tubules of rats appear to reabsorb a constant fraction of the phosphate filtered by glomeruli. In earlier work, Wolf and Ball[10] were unable to show an effective renal threshold for calcium retention in the dog. Knapp[8] has examined data from a large number of balance studies conducted with humans and concluded that the level of output of calcium in the urine is largely an exponential function of the dietary calcium intake, expressed on a body-weight basis. The calcium excretion in the urine, therefore, like calcium absorption, is probably not the limiting process in calcium metabolism.

The role of the *feces* in calcium homeostasis has a long history of controversy (see Nicolaysen[11] for an extensive review of the earlier literature, and Steggerda and Mitchell[12] for a briefer critical review of the later literature on this subject). Neither McCance and Widdowson[13] nor Baylor *et al.*[14] were able to demonstrate increased fecal output of calcium following intravenous injection of relatively massive doses of calcium. The conclusion was therefore drawn that the feces do not represent a significant route of excretion for endogenous calcium. A large number of experiments with both humans[6, 15-17] and animals[18-27] has shown that when Ca^{45} is injected systemically the intestinal contents become labeled very rapidly, and that some of the Ca^{45} in the intestinal tract is lost in the stools. Evidently there are species differences in the way endogenous calcium is partitioned between urine and feces,[6] but there can be little doubt now that human beings, as well as all animals studied, excrete endogenous calcium via the feces.

Nicolaysen's[11] detailed observations on dogs are consistent with the hypothesis that endogenous calcium lost in the feces is calcium that has escaped absorption. This hypothesis finds support in the work of Thomas and collaborators[28] who observed increased fecal output of calcium in rabbits fed sodium versenate by stomach tube, followed at once by intravenous injection of Ca^{45}. Steggerda and Mitchell[12] made somewhat similar observations on humans on a diet containing large quantities of citrate in the form of orange juice. In both cases it is to be presumed that the calcium that entered the lumen of the intestine via the succus entericus and the bile formed nonabsorbable calcium complexes, thereby raising fecal calcium excretion.

While these considerations indicate that the feces are a significant route for the excretion of endogenous calcium, they do not indicate that the output of endogenous calcium is the limiting process of calcium metabolism.

When Ca^{45} is injected, all of the fecal Ca^{45} is endogenous. The *quantity of endogenous calcium excreted in the feces* can then be calculated with the aid of the following equation:

$$\overline{S.A.}_{s} = A_f/En_f \qquad (1)$$

where $\overline{S.A.}_{s}$ = mean specific activity of serum between time 0 and time t

A_f = total Ca^{45} recovered in the feces by time t

and En_f = total endogenous calcium excreted in the feces by time t

TABLE 2 lists the quantity of endogenous calcium excreted by several of our subjects.* These values were obtained by determining the mean serum specific activity by graphical integration, and dividing this value into the total

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* When a corrected value of the mean serum specific activity of subject no. 57 was used to recalculate his output of endogenous calcium in the feces, the value shown in TABLE 2 resulted, and not the value of 15 percent reported earlier.[6]

TABLE 2

FECAL OUTPUT OF ENDOGENOUS CALCIUM

Subject no.	Age (yrs.)	Endogenous calcium*		Period (hours)
		mg.	% of total fecal Ca	
10	11.2	336	10.5†	77
14	10.9	425	9.9	97
15	15.7	239	5.3	100
16	13.1	328	6.0	97
17	12.7	355	11.4	97
18	11.6	186	5.4	97
57	21.0	500	8.3	103

* Calculated on basis of EQUATION 1.

† Fecal collection of day 1 incomplete. Figure in table based on projected Ca output; true percentage: 15.5.

fecal Ca^{45} output in that period of time. The range of these values is consistent with those reported by Blau and his co-workers.[17] It is also in accord with the value Brine and Johnston[22] calculated by extrapolating to an intake of zero a plot of the fecal output against calcium intake.

Transport

The enormous speed with which injected Ca^{45} leaves the bloodstream does not make it appear likely that calcium transport in the blood is a limiting process in any way. Several workers[15, 33, 49, 51] have shown that the serum calcium level is raised only temporarily in calcium load tests, much of the injected calcium being rapidly excreted in the urine. This again suggests that the blood can readily dispose of the amounts of calcium normally absorbed from the gastrointestinal tract. Howard[32] has also discussed this point in detail.

Bone Salt Formation and Resorption

Bauer and his collaborators[5] have recently proposed a procedure for calculating the rate of bone formation, often called "accretion." The procedure makes use of data obtained from metabolic studies carried out with the aid of radioisotopes. Bauer and his collaborators state that the quantity of isotope in the skeleton at any moment is the sum of exchangeable and nonexchangeable (that is, accreted) fractions, minus any quantity resorbed. If it is assumed that, following injection of Ca^{45}, the specific activity of the exchangeable calcium in the skeleton very rapidly equilibrates with that of the serum, then the quantity of exchangeable Ca^{45} in the skeleton at any time t equals the specific activity of the blood calcium at time t multiplied by the quantity of exchangeable calcium, E, in the skeleton. The quantity of accreted calcium will equal the mean specific activity between time 0 and time t multiplied by the accretion rate, A, and the time elapsed.

If the quantity resorbed is considered negligible in a short term experiment (for example, 5 days in a study with humans) the relationship proposed by Bauer *et al.*[5] can be written as follows:

$$Q_t = E(S.A._t) + A \int_0^t (\overline{S.A._s})\, dt \qquad (2)$$

where Q_t = quantity of Ca^{45} in skeleton at time t following injection at time 0
E = quantity of exchangeable calcium in skeleton
$S.A._t$ = serum specific activity at time t
$\overline{S.A.}_s$ = mean serum specific activity between time 0 and time t, and
A = rate of calcium accretion.

Q_t can be determined experimentally in animals. In human studies Q_t is taken as the quantity of Ca^{45} retained in the body at time t, that is, the quantity of isotope given minus the quantity excreted in urine and feces. The equation therefore has two unknowns: E, a term denoting a quantity; and A, denoting a rate. It can be solved by measuring the specific activity of the serum at two different times and by substituting these values in the equation. Since excretion of calcium is discontinuous, particularly in the stools, a retention curve is constructed that has the same course as the serum specific activity with time (see FIGURE 2). The mean serum specific activity value is also obtained from the serum specific activity curve. Note that the accretion term of EQUATION 2 is based on reasoning similar to that by which the endogenous fecal calcium output was calculated (EQUATION 1).

To be valid, EQUATION 2 requires that the interval t be chosen small enough so that resorption is truly negligible. The equation also implies that no calcium compartment communicates with the skeleton except via the serum.

While this approach may be theoretically imperfect, it seemed of interest to calculate the rate of bone salt accretion (A), and the quantity of exchangeable bone calcium for some of our subjects (TABLE 3).*

TABLE 3
SIZE OF EXCHANGEABLE FRACTION OF BODY CALCIUM (E) AND RATE OF BONE SALT ACCRETION (A), CALCULATED ACCORDING TO BAUER, CARLSSON, AND LINDQUIST[5]

Subject	Age yrs.	E		A
		gm.	% of estimated body Ca*	gm. Ca/day
10	11.2	6.9	1.06	2.1
11	12.6	5.9	0.90	2.1
12	12.4	6.2	0.82	2.1
13	13.1	5.8	0.81	2.3
15	15.7	6.7	0.82	2.8
16	13.1	6.4	0.81	2.1
17	12.7	6.7	0.92	1.8
18	11.6	6.2	0.99	2.7
55	12.6	11.0	1.76	2.3
Mean	12.8	6.9	0.99	2.1
56	22.0	3.9	0.39	0.86
57	21.0	5.5	0.53	0.73

* Body calcium estimated as 2 per cent of body weight.

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* While in this country recently, A. Carlsson and G. C. H. Bauer very kindly calculated these values for us.

The agreement of the values for subjects of comparable ages studied at different times is gratifying. The differences between the adults and the boys are striking, particularly because the values were calculated for each subject independently.

The calcium intake of the boys during the course of these experiments approximated 2 gm. per day, of which they absorbed at most 1 gm. (see FIGURE 3). Bone salt, therefore, was formed at a rate 2 to 3 times the calcium intake, and bone salt resorption must have occurred at a rate no less than 1 gm. per day. In the adults the corresponding rates were lower.

On the basis of the preceding considerations it seems reasonable to think that either bone salt accretion or resorption is the slowest process of calcium metabolism. Our data on human subjects do not tell which it is. Possibly both are limiting. Carlsson[33] has suggested that resorption, rather than accretion, is the homeostatic process. In that case a decreased calcium intake would result in increased resorption of bone mineral. If this attractive hypothesis is verified experimentally, it would mean that bone salt accretion is the limiting process.

Summary

(1) Some aspects of calcium metabolism in human beings have been studied quantitatively with the aid of radioactive calcium (Ca^{45}). The disappearance of injected Ca^{45} from the blood of 9 adolescent boys and 1 male adult over a period of 5 days can be described by exponential equations with 4 terms. The half-times of the last 3 terms of these equations are appreciably higher for the adult than for the boys. Viewed together with higher retention values for the boys, the longer half-times for the adult suggest a lower rate of bone formation.

(2) Studies with 20 boys and 1 other adult showed that absorption varied little with age, but markedly with intake. As calcium intake increased, the per cent absorbed decreased.

(3) The fecal route was shown to be significant for the excretion of endogenous calcium. In the 7 subjects studied, from 5 to 15 per cent of the fecal calcium output was endogenous.

(4) It is suggested that bone salt formation and resorption are rate-limiting processes in a scheme of calcium metabolism that involves absorption, transport, bone salt formation, bone salt resorption, and excretion.

Acknowledgments

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References

1. NATIONAL RESEARCH COUNCIL. FOOD AND NUTRITION BOARD. 1948. Recommended daily dietary allowances. Rev. ed. (Reprint and circular series No. 129) National Research Council, Washington, D. C.

2. Salomon, K., B. W. Gabrio & G. F. Smith. 1946. A precision method for the quantitative determination of calcium in blood plasma. Arch. Biochem. **11:** 433–443.
3. Bronner, F., R. S. Harris, C. J. Maletskos & C. E. Benda. 1954. Studies in calcium metabolism. Effect of food phytates on calcium^{45} uptake in children on low-calcium breakfasts. J. Nutrition. **54:** 523–542.
4. Tomlin, D. H., K. M. Henry & S. K. Kon. 1955. The interstitial metabolism of calcium in the bones and teeth of rats. Brit. J. Nutrition **9:** 144–156.
5. Bauer, G. C. H., A. Carlsson & B. Lindquist. 1955. Evaluation of accretion, resorption and exchange reactions in the skeleton. Kungl. Fysiografiska Sallskapets i Lund Forhandlinger. **25**(1): 1–16.
6. Bronner, F., R. S. Harris, C. J. Maletskos & C. E. Benda. 1956. Studies in calcium metabolism. The fate of intravenously injected radiocalcium in human beings. J. Clin. Invest. **35:** 78–88.
7. Flexner, L. B., D. B. Cowie & G. J. Vosburgh. 1950. Studies on capillary permeability with tracer substances. : 80–93. Brookhaven symposia in biology. No. 1 (Conference on Biological Applications of Nuclear Physics, July 12–27, 1948). Technical Information Service (BNL-C-4). Oak Ridge, Tenn. : 80–93.
8. Knapp, E. L. 1947. Factors influencing the urinary excretion of calcium. I. In normal persons. J. Clin. Invest. **26:** 182–202.
9. Crawford, J. D., D. Gribetz, N. B. Talbot, M. Terry, E. A. MacLachlan, K. Van Loon & M. F. Morrill. 1955. Mechanisms of tubular phosphate reabsorption and the influence thereon of vitamin D in completely parathyroidectomized rats. Am. J. Physiol. **180:** 156–162.
10. Wolf, A. V. & S. M. Ball. 1949. Effect of intravenous calcium salts on renal excretion in the dog. Am. J. Physiol. **158:** 205–207.
11. Nicolaysen, R. 1934. Untersuchungen über die Kalkauscheidung bei Hunden. Ein Beitrag zur Physiologie des Kolons. Skand. Arch. Physiol. Suppl. **69:** 1–66.
12. Steggerda, F. R. & H. H. Mitchell. 1946. The effect of the citrate ion on the calcium metabolism of adult human subjects. J. Nutrition. **31:** 423–438.
13. McCance, R. A. & E. M. Widdowson. 1939. The fate of calcium and magnesium after intravenous administration to normal person. Biochem. J. London. **33**(1): 523–529.
14. Baylor, C. H., H. E. Van Alstine, E. H. Keutmann & S. H. Bassett. 1950. The fate of intravenously administered calcium. Effect on urinary calcium and phosphorus, fecal calcium and calcium-phosphorus balance. J. Clin. Invest. **29:** 1167–1176.
15. Bellin, J. & D. Laszlo. 1952. Metabolism and removal of Ca^{45} in man. Science. **117:** 331–334.
16. Geissberger, W. 1952. Die Calciumresorption und-Retention beim Menschen nach intravenöser, oraler und rectaler Calciumverabreichung mit Bilanzen unter Anwendung von radioaktivem Calcium. Z. ges. exptl. Med. **119:** 111–142.
17. Blau, M., H. Spencer, J. Swernov & D. Laszlo. 1954. Utilization and intestinal excretion of calcium in man. Science. **120:** 1029–1031.
18. Lienke, R. I., G. Cullen & W. D. Armstrong. 1949. Studies on the excretion and distribution of radioactive calcium. Conference on Metabolic Interrelations. 1st Conference, New York, N. Y., Transactions. Josiah Macy, Jr. Foundation, New York, N. Y. : 73–76.
19. L'Heureux, M. V., W. R. Tweedy & E. M. Zorn. 1949. Excretion of radiocalcium by normal rats. Proc. Soc. Exptl. Biol. Med. **71:** 729–732.
20. Harrison, H. E. & H. C. Harrison. 1950. The uptake of radiocalcium by the skeleton: The effect of vitamin D and calcium intake. J. Biol. Chem. **185:** 857–867.
21. Singer, L. & W. D. Armstrong. 1951. Retention and turnover of radiocalcium by the skeleton of large rats. Proc. Soc. Exptl. Biol. Med. **76:** 229–233.
22. Wallace, H. D., R. L. Shirley & G. K. Davis. 1951. Excretion of Ca^{45} into the gastrointestinal tract of young and mature rats. J. Nutrition. **43:** 469–475.
23. Shirley, R. L., J. C. Driggers, J. McCall, G. K. Davis & N. R. Mehrhof. 1941. Excretion and retention of P^{32} and Ca^{45} by laying hens. Poultry Sci. **30:** 730–734.
24. Thomas, R. O., T. A. Litovitz, M. I. Rubin & C. F. Geshickter. 1952. Dynamics of calcium metabolism; time distribution of intravenously administered radiocalcium. Am. J. Physiol. **169:** 568–575.
25. Armstrong, W. D., J. A. Johnson, L. Singer, R. J. Lienke & M. L. Premer. 1952. Rates of transcapillary movement of calcium and sodium and of calcium exchange by the skeleton. Am. J. Physiol. **171:** 641–650.
26. Hansard, S. L., C. L. Comar & M. P. Plumlee. 1952. Absorption and tissue distribution of radiocalcium in cattle. J. Animal Sci. **11:** 524–535.

27. Hansard, S. L. & M. P. Plumlee. 1954. Effects of dietary calcium and phosphorus levels upon the physiological behavior of calcium and phosphorus in the rat. J. Nutrition. **54:** 17–31.

28. Thomas, R. O., T. A. Litovitz & C. F. Geschickter. 1954. Alterations in dynamics of calcium metabolism by intraintestinal calcium reservoirs. Am. J. Physiol. **176:** 381–387.

29. Brine, C. L. & F. A. Johnston. 1955. Endogenous calcium in the feces of adult man and the amount of calcium absorbed from food. Am. J. Clin. Nutr. **3:** 418–420.

30. Schilling, A. & D. Laszlo. 1951. Rate of urinary calcium excretion following its intravenous administration as an indicator of bone metabolism. Proc. Soc. Exptl. Biol. Med. **78:** 286–289.

31. Meiselas, L. E. & M. G. Goldner. 1955. Failure of calcium tolerance test as a diagnostic aid in malignant osteolytic disease. Metabolism. **4:** 250-257.

32. Howard, J. E. 1954. Normal calcium and phosphorus transport and body fluid homeostasis. In Conference on Metabolic Interrelations, with Special Reference to Calcium. Fifth Conference, New York, 1953. Transactions. Josiah Macy, Jr. Foundation. New York, N. Y. : 11–42.

33. Carlsson, A. 1951. Metabolism of radiocalcium in relation to calcium intake in young rats. Acta Pharmacol. Toxicol. **7:** Suppl. 1.

Part II. Parathyroid Hormone

STUDIES ON THE MAINTENANCE OF SERUM CALCIUM LEVELS BY PARATHYROID ACTION ON BONE AND KIDNEY

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The importance of the parathyroid glands in the maintenance of a constant plasma concentration of calcium ions has long been recognized. The means by which the hormone of the parathyroid gland accomplishes this task has been the subject of considerable controversy over the past 20 years. Even today it is possible to read or hear talks that, on the one hand, attribute the action of the hormone entirely to an influence on bone and, on the other hand, consider the kidney as the only target organ. It is the purpose here, of myself and my associates in this work, to suggest that the parathyroids control serum calcium levels by coordinating the effects on bone with those on the kidney into a system that not only is the most efficient for maintaining the level within narrow limits, but that performs this task with a minimum amount of manipulation of calcium ions. We make no claim that any of our ideas are new. While we hope to back them up with experimental data from our own laboratories, they can also be gleaned from the literature dating back to the late twenties (for general review, see Greep and Kenny, 1955; for extrarenal effect, see Grollman, 1954; for calcium excretion, Jahn and Pitts, 1948).

There are 3 points especially to be emphasized in this report:

(1) While the physiological purpose of the gland is to control calcium levels, there must also necessarily, because of the close tie between the 2 ions in the body, be effects on phosphate metabolism. While the effects on phosphate are not considered to be the cause of the control of calcium levels, however, they could conceivably be the cause of major calcium removal from bone. This apparent contradiction will be explained later.

(2) Is the mechanism as postulated found in all species? Our experimental data is confined to the rat. A possible parallel in other mammals, however, will be discussed.

(3) The parathyroids control not only calcium metabolism of bone, but also calcium excretion by the kidney. In regard to this control of the calcium ion there follows a very simple illustration, but one that explains the calcium control as we believe it to exist in the body, and that also demonstrates clearly the efficiency of such a system for accurate maintenance of specified levels. The comparison is to a lake contained at its lower end by a dam with controllable gates and fed by two rivers, one a large, long, deep river, the other a mountain stream fluctuating with daily rainfall. To maintain the level of the water in the lake accurately and efficiently at all times, one would have to control both the gates of the dam and the flow of water from the rivers, for to lower the gates while the water was running fast would allow a continuous large loss of water over the dam for a relatively small decrease in the level of the lake. On

the other hand, raising the gates while the river was low would permit a long lag before the desired new level was achieved. A simultaneous change in the dam gate and the river flow, however, would permit continuous accurate control of the water level in the lake. This example illustrates the way we believe the bone and kidney work together to maintain, within such narrow limits, the calcium ion concentration of the extracellular spaces.

In presenting the experimental data from our laboratory in support of these theses, we have taken the liberty of including only illustrative actual experiments and, for the most part of summarizing the work by diagrammatic presentation. Our work has been limited almost entirely to the parathyroidectomized rat. We have emphasized parathyroidectomy instead of the injection of extract into normal animals, for we feel that by this means we are working within physiological levels. The extract, when administered, could therefore be reduced to less than 10 IU per animal, and often to as little as 3 or 4 IU. In addition, by the study of the effects of parathyroidectomy and the recovery by injection, we reduce to a minimum the problem of the effects of an extract that, at its best, is considered crude.

Our first experimental evidence is in support of the thesis that the hormone of the parathyroid lowers the kidney threshold for phosphate excretion while simultaneously raising the threshold for calcium excretion. The data stated in TABLE 1 gives a typical experiment illustrating this contention. For these experiments the animals were parathyroidectomized individually under ether anesthesia. One hour later these, along with sham-operated controls, were given a 5 ml. water dose and placed in metabolism cages for urine collection. Analyses were made on urine samples collected over varying intervals of time (for more detailed experiments and for description of methods, see Talmage and Kraintz, 1954; and Talmage *et al.*, 1955).

It will be noted that by the second hour after parathyroidectomy there was a sharp drop in phosphate excretion and a marked elevation in calcium excretion. The phosphate excretion persisted low for about 18 hours, at which time it rose to within normal levels. The calcium excretion jumped within the first hour and gradually dropped thereafter, reaching the normal range at approximately the same time as did the phosphate excretion. When the phos-

TABLE 1
PROGRESSIVE CHANGES IN CALCIUM AND PHOSPHATE EXCRETION FOLLOWING PARATHYROIDECTOMY

	Groups	Hours after parathyroidectomy					
		2	2–4	7–10	13–16	19–22	25–28
Phosphate excretion (mg. P/hr.)	Normal	.40 ± .05	.61 ± .09	.76 ± .12	1.3 ± .21	1.0 ± .17	.73 ± .24
	PTX	.02 ± .005	.04 ± .02	.02 ± .01	.01 ± .01	1.23 (3)	1.16 ± .14
Calcium excretion (mg./hr.)	Normal	.055 ± .025	.045 ± .009	.025 ± .004	.019 ± .001	.019 ± .002	.018 ± .001
	PTX	.178 ± .024	.289 ± .060	.092 ± .018	.040 ± .011	.031 ± .009	.029 ± .006

All groups include 4 or more animals except where number of animals is given in parentheses. Values given with standard error of mean.
PTX = parathyroidectomized.

phate excretions of a group of animals are averaged, it might appear that the return to within normal range is gradual. If the individual animals are followed, however, it will be found that for any one animal the reversal toward the normal range occurred within as short a time and was as dramatic as was the initial drop. A diagrammatic presentation of the sequence of events following parathyroidectomy is given in FIGURE 1. It should be noted that during the interval of time shown in this graph the plasma level for phosphate was rapidly rising, reaching a value of approximately 13 mg. P per 100 ml. at the time of reversal toward the normal excretion. At the same time, the plasma calcium level was rapidly falling, and reached a value of somewhat less than 7 mg. per 100 ml. at the time normal excretion values for calcium reappeared. Since the phosphate excretion value was low in the presence of a rising plasma value and, since the calcium excretion was high in the presence of a dropping plasma value, the only conclusion that seems valid is that these excretion changes were due to renal effects and could not be attributed to extrarenal influences.

It is our contention that the excretion changes are due to sudden changes in the renal thresholds for calcium and phosphate, and that when the plasma values have equilibrated with the new thresholds, excretion values within the normal range reappear. Because the phosphate changes are dramatic, both in the drop and the subsequent rise, this hypothesis is easily tested for phosphate excretion. FIGURE 2 presents these ideas diagrammatically, suggesting that the return of normal excretion occurs when the plasma phosphate level

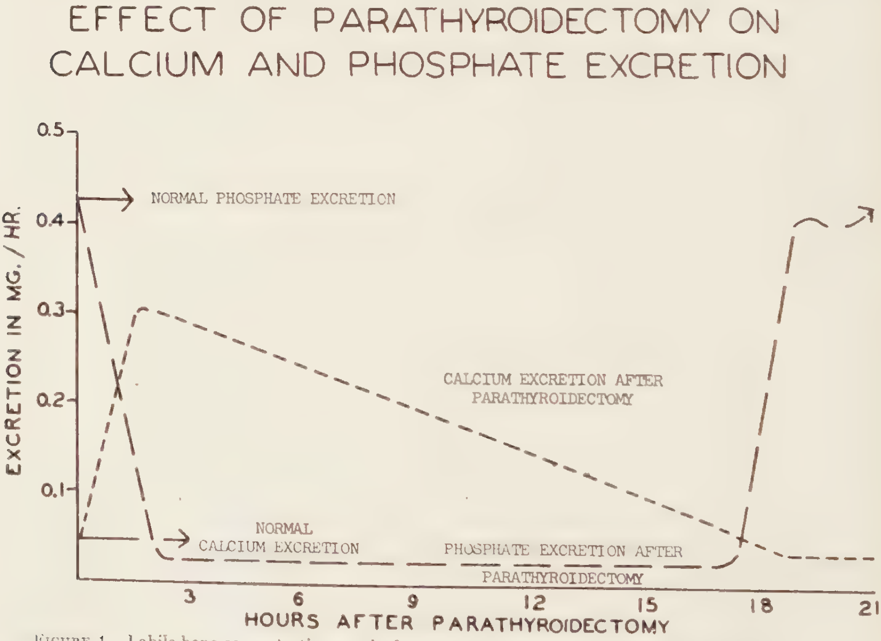

FIGURE 1. Labile bone concentrations equivalent to ionic concentration in 100 ml. fluid media.

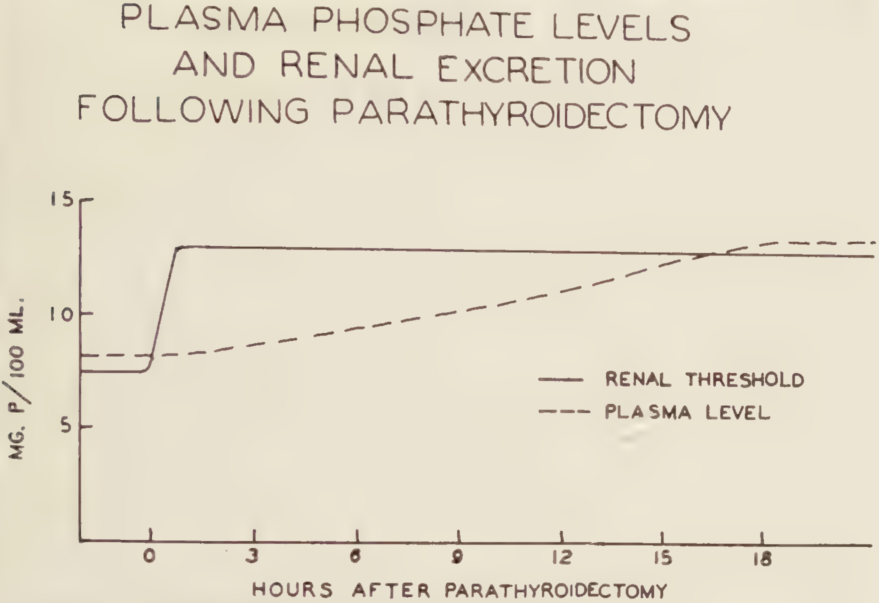

FIGURE 2. PO_4 concentrations given in mg. phosphorus per 100 ml.

has risen to match the new threshold of 13 mg./100 ml. In support of this, it can be shown that if 1.5 mg. P as phosphate is injected hourly commencing 1 hour after parathyroidectomy, the only change is a shortening to 9 hours of the time at which the reversal occurs, the plasma value at the time of reversal still remaining in the vicinity of 13 mg. P per 100 ml. If, however, the 1.5 mg. per hour of phosphorus is given commencing after the reversal toward the normal excretion values has occurred, there is an immediate increase in phosphate excretion, significant by the first hour. On the other hand, if as little as 1 IU of parathyroid extract is administered per hour starting 1 hour after parathyroidectomy, the reversal also occurs at about 9 hours after parathyroidectomy, but is accompanied by a decrease in plasma phosphate levels. If the extract is administered after the reversal occurs, the increase in phosphate excretion can be detected within 1 hour, accompanied by a gradual drop in plasma phosphate values. We feel that experiments of this type, added to the bulk of experimental work of others, make it imperative that a direct action of the parathyroids on renal function be postulated.

For our bone studies we have utilized nephrectomized rats maintained by peritoneal lavage. In these studies the kidneys were removed by a ventral incision, and a plug designed by Kloff and Page (1954) stitched into the incision. The rats were parathyroidectomized 24 hours after nephrectomy to allow the animals to go through the transitory fluctuations in calcium levels that have been noted following nephrectomy (Elliott and Freeman, 1955). Three times daily 30 ml. of an isotonic wash solution, but void of both phosphate and calcium, was placed in the peritoneal cavity and allowed to equilibrate for 1 hour before removal. While analyses of other ions were performed, this discussion will be confined only to phosphate and calcium ion changes. Our animals were maintained on a limited diet, which in several experiments was extended to a calcium-free diet for at least 3 days prior to experimental use. While the involvement of the bone in these experiments is purely circumstantial, modern concepts of bone metabolism make this conclusion well-warranted (for elaboration of methods used and more detailed results, see Talmage and Elliott, 1956).

The results of a typical experiment are shown in TABLE 2. There are several points to be emphasized. The first is the consistency of the calcium levels of the equilibrated wash, both in control and parathyroidectomized rats. Parathyroidectomy reduced the calcium ion concentration almost in half, but at that point it was maintained throughout the experiment. Since a calcium-free wash was inserted in all instances this illustrates the speed of equilibration of the extracellular fluid spaces with bone. It appears to us to be another clear demonstration of the "feed-back" system proposed by McLean and Urist (1955). A second point is that the changes in phosphate ion concentration always parallel the changes in calcium. Despite the gradual accumulation of phosphate from extrarenal sources, parathyroidectomy produced an immediate drop of the same magnitude as the calcium drop. A third point is the ease with which as little as 1 IU of parathyroid extract administered hourly for 6 hours is able to raise both the calcium and phosphate concentrations. For the fourth point, reference is made to TABLE 3. In regard to the discussed renal changes it has been pointed out that calcium and phosphate changes could be detected within 1 hour. It was of interest to determine whether the same time was sufficient for the bone changes. For this experiment, the first wash was placed in the rats one-half hour after parathyroidectomy and removed at 1 hour. A second wash was then immediately placed in the peritoneal cavity and allowed to equilibrate for the usual hour. It can be noted that the calcium drop was already significant by the end of the first hour while, because of the greater variability in phosphate values, this drop was not signif-

TABLE 2

PERITONEAL WASH ANALYSIS IN NEPHRECTOMIZED RATS FOLLOWING PARATHYROIDECTOMY

(Each Group Includes 5 or More Animals—Values Given with S.E.)

Experimental condition	Groups	PO_4 (Mg. P/100 ml.)	Ca Mg./100 ml.
24 hours postnephrectomy		8.8 ± .4	6.1 ± .1
28 hours postnephrectomy; 4 hours postparathyroidectomy	Controls	10.9 ± .6	6.5 ± .2
	Parathyroidectomized	8.3 ± .4	3.9 ± .2
48 hours postnephrectomy; 24 hours postparathyroidectomy	Controls	12.4 ± .9	6.8 ± .1
	Parathyroidectomized	9.5 ± .6	3.7 ± .2
54 hours postnephrectomy; 30 hours postparathyroidectomy; 6 hours parathyroid treatment—1 IU/hr. to parathyroidectomized animals.	Controls	9.9 ± .1	5.6 ± .1
	Parathyroidectomized	7.5 ± .7	3.7 ± .3
	Parathyroid treated	8.7 ± .9	5.4 ± .3

* S. E. = standard error.

TABLE 3

PERITONEAL WASH ANALYSIS IN NEPHRECTOMIZED RATS 1 TO 2 HOURS FOLLOWING PARATHYROIDECTOMY

(Each Group Includes 5 or More Animals Values Given with S.E.)

Experimental condition	Groups	PO_4 (Mg. P/100 ml.)	Ca (Mg./100 ml.)
24 hours postnephrectomy		11.8 ± .2	6.7 ± .1
24 hours postnephrectomy; 30–60 min. postparathyroidectomy	Controls	7.7 ± .4	4.2 ± .2
	Parathyroidectomized	7.2 ± .5	3.5 ± .1
25 hours postnephrectomy; 1–2 hours postparathyroidectomy	Controls	9.0 ± .4	5.2 ± .2
	Parathyroidectomized	7.4 ± .1	3.5 ± .1

icant until the second hour. The speed with which these changes occur would seem to negate any need for cellular changes to occur to permit the action of the parathyroid hormone in physiological doses on bone metabolism. These experiments, along with many experiments carried out in nephrectomized animals by other investigators are, in our opinion, absolute proof of the direct action of the parathyroids on bone.

During the course of these experiments on nephrectomized animals it soon became obvious that parathyroidectomy was not followed by muscular spasms or any other sign of tetany even in animals parathyroidectomized for 3 days and maintained for up to 7 days on a calcium-free diet. This is in marked contrast to the work from our own laboratory, in which it was normal for parathyroidectomized rats with intact kidneys to show signs of muscular spasms as early as 9 hours after removal of the parathyroids. In addition, approximately half of our parathyroidectomized rats died in tetany within 24 hours. This is in agreement with the work of Munson and his collaborators (1955), who reported death from tetany as early as 9 hours in rats maintined prior to use on calcium-free diets.

Another series of experiments was therefore run, in which animals were treated identically except that the kidneys were left intact in some of the groups. Invariably, animals with kidneys intact showed muscular spasms between 6 and 9 hours after parathyroidectomy. When washed with calcium-free wash, however, the spasms subjectively appeared to be slightly alleviated, and the wash on equilibrium showed calcium concentrations similar to that of the nephrectomized-parathyroidectomized animals. It appeared that the kidney threshold might be lower than the bone extracellular fluid equilibration level. Following the addition of the large volume to the extracellular space, however, the bone equilibration occurred long before the kidney could reduce the volume and the calcium concentrations. The experiment was repeated, therefore, and, at 24 hours after parathyroidectomy, the animals were bled without further lavage. It was observed that the plasma calcium of the animals undergoing muscular spasms was lower than that of their nephrectomized-parathyroidectomized controls. The differences, however, though significant, were small. While more work needs to be done on this point, the existing evidence leads us to suspect that, following parathyroidectomy, the kidney threshold for calcium very probably falls sufficiently below the level of bone extracellular fluid equilibrium to permit the animal on low calcium intake to start the tetany syndrome.

As a final correlation of the ideas presented in this paper, we are presenting 3 diagrammatic representations of the parathyroid control of calcium and phosphate equilibria as visualized by our laboratory. In the first of these, FIGURE 3, is shown the calcium and phosphate ion balance between bone, plasma, and kidney threshold. The value used for the calcium ion concentration of the labile fraction of bone is that determined by our experiments as equivalent to 6 mg. per 100 ml. in a fluid media. Since the kidney threshold is in the same range, there is a marked equilibrium in calcium ion concentration in these 3 phases. The phosphate ion concentration of labile bone is determined by the approximate ratio of 1:5 for calcium to phosphorous in bone. The kidney threshold for phosphate in the normal rat is considerably higher so that, despite the low level of phosphate ion available in labile bone, the plasma concentration of phosphate is approximately 7.5 mg. P per 100 ml. It is of interest to point out that in man, with the kidney threshold set considerably lower than in the rat, the phosphate ion concentration of these 3 phases is almost as constant as is the calcium.

The effect of parathyroidectomy upon this equilibrium is illustrated in FIGURE 4. The equilibrium between stable and labile bone is reduced to a value equivalent to 3.5 mg. per 100 ml. for calcium and to a calculated 2.5 mg. P per 100 ml. for phosphate. In the nephrectomized rat the calcium ion concentration of the plasma is in equilibrium with the bone. With the kidneys intact, however, due to the lower threshold than this equilibrated value, the plasma concentration may drop sufficiently to cause the animal to go into tetany. In the parathyroidectomized animal, because of the raised kidney threshold for phosphate, the plasma levels rise far above the equilibrium level set by the labile bone.

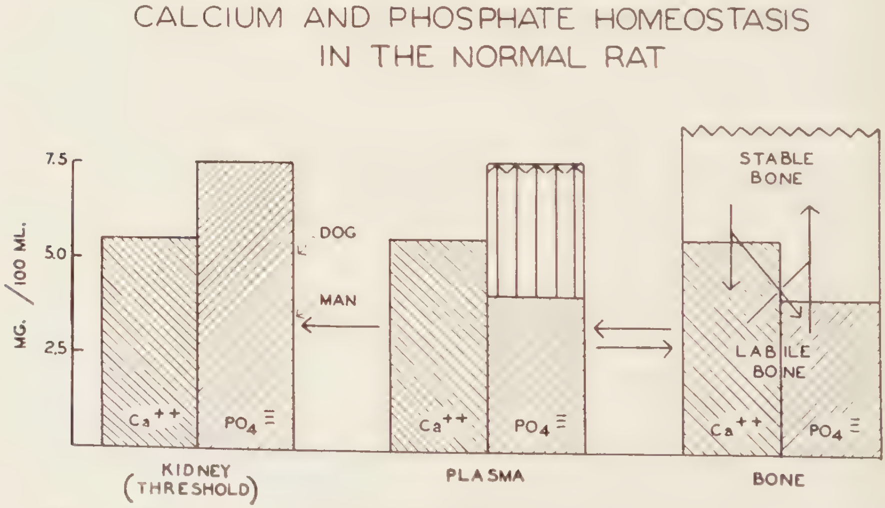

FIGURE 3. Kidney threshold values given in mg. per 100 ml.

FIGURE 5 presents the diagrammatic picture of the postulated parathyroid control of calcium and phosphate in the rat. It is generally assumed, though not conclusively proved, that fluctuations in the ionic calcium concentration of the plasma trigger the activity of the parathyroids. An increase in the output of the gland raises the labile bone fraction of calcium and phosphate

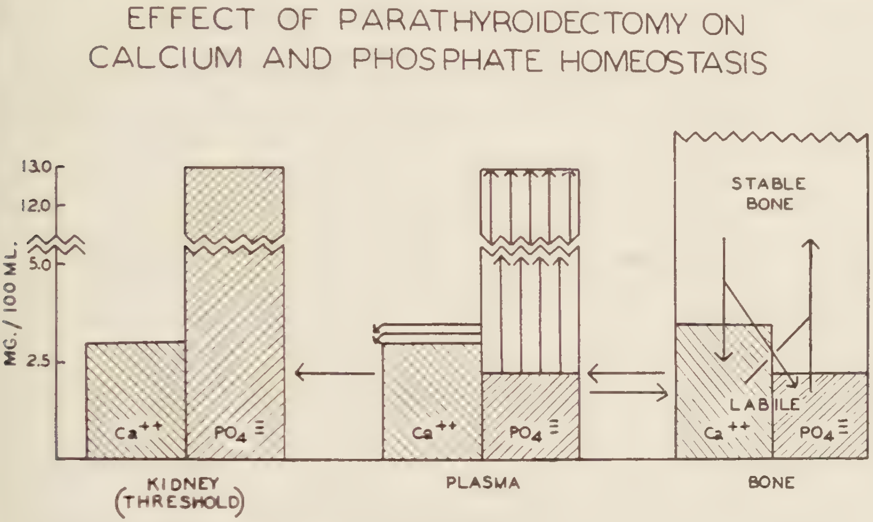

FIGURE 4. Plasma calcium values refer only to the diffusible (primarily ionized) calcium of the plasma.

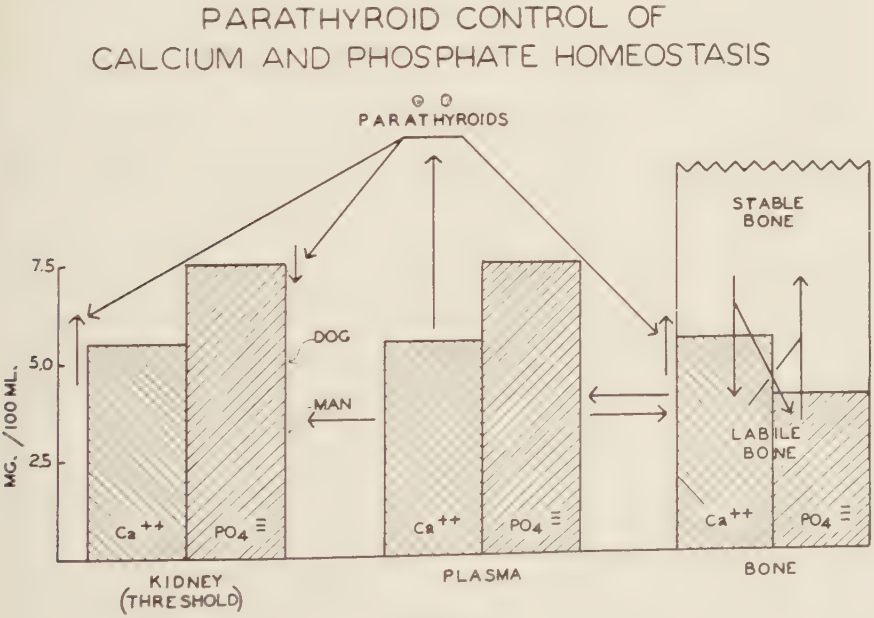

FIGURE 5.

ions. Simultaneously, the kidney threshold for calcium is raised, reducing to a minimum the amount of calcium needed to adjust the plasma levels. With such a system in effect, the amount of calcium necessary to double the plasma calcium levels would probably be less than 0.1 per cent of the total calcium in bone.

According to our postulate, the hormonal effect on phosphate is to raise simultaneously the labile bone phosphate level while lowering the kidney threshold for phosphate excretion. Under normal physiological conditions this effect on phosphate is relatively insignificant and plays no part in calcium control or in the over-all economy of calcium utilization. If, however, under conditions of hormonal treatment or over-secretion of the parathyroids, the phosphate threshold is pushed below the rising phosphate level of labile bone, a completely new sequence of events is brought into play. This low threshold for phosphate produces a "one-way flow" of phosphate permitting marked excretion of this ion and pulling phosphate from the labile bone component. This would automatically pull calcium out of this component also and, because of the equilibrium level, both of these ions would be immediately resupplied from stable bone. This calcium lost from the bone would do little to raise the plasma calcium level, but would be immediately lost via the kidney. Such a condition of secondary removal of calcium from bone would be most easily achieved in a species such as man where the kidney threshold for phosphate excretion, in the normal animal, is in close equilibrium with the bone fraction, and it would be progressively harder to produce in the dog and the rat, where the normal phosphate renal threshold is correspondingly higher. It would be logical to assume, therefore, that in man the increased phosphate excretion is actually the primary cause of hyperparathyroid bone disorders.

In summary, we have attempted to postulate a control of plasma calcium levels by the parathyroids that necessitates the minimum amount of manipulation of calcium ions. Under physiological conditions the effects on phosphate metabolism have little bearing on this control. Under pathological conditions, however, it may be the increased excretion of phosphate by the kidney that leads to the pronounced loss of calcium and phosphate from bone.

Acknowledgments

This work, from its instigation, has been supported in part by Contract AT-(40-1)-284 with the Atomic Energy Commission. The author acknowledges the cooperation and collaboration of F. W. Kraintz, R. D. Barnes, G. D. Buchanan, R. F. Frost, and J. R. Elliott during various stages of the work. The technical assistance of Donna Johnson has been appreciated.

References

Elliott, J. R. & S. Freeman. 1955. Kidney parathyroid relation to plasma citric acid and calcium. Fed. Proc. **14:** 43.

Greep, Roy O. & A. D. Kenny. 1955. Physiology and chemistry of the parathyroids. in *The Hormones*. **3:** Pincus and Thimann, Academic Press. New York, N. Y.

Grollman, A 1954. The role of the kidney in the parathyroid control of the blood calcium as determined by studies on the nephrectomized dog. Endocrinology. **55:** 166.

Jahn, I. & R. F. Pitts. 1948. Effects of parathyroid on renal tubular reabsorption of phosphate and calcium. Am. J. of Physiol. **155:** 42.

KOLFF, W. J. & I. H. PAGE. 1954. Influence of protein and other factors on postnephrectomy hypertension in rats sustained with an improved method of peritoneal lavage. Am. J. Physiol. **178:** 69.

McLEAN, F. C. & M. R. URIST. 1955. *Bone*. University of Chicago Press. Chicago, Ill.

MUNSON, P. 1955. Studies on the role of the parathyroids in calcium and phosphorus metabolism. Ann. N. Y. Acad. Sci. **60**(5): 776.

TALMAGE, R. V. & J. R. ELLIOTT. 1956. Changes in extracellular fluid levels of calcium, phosphate, and citrate ions in nephrectomized rats following parathyroidectomy. Endocrinology. In press.

TALMAGE, R. V. & F. W. KRAINTZ. 1954. Progressive changes in renal phosphate and calcium excretion in rats following parathyroidectomy or parathyroid administration. Proc. Soc. Exptl. Biol. Med. **87:** 263.

TALMAGE, R. V., F. W. KRAINTZ & G. D. BUCHANAN. 1955. Effect of parathyroid extract and phosphate salts on renal calcium and phosphate excretion after parathyroidectomy. Proc. Soc. Exptl. Biol. Med. **88:** 600.

THE INFLUENCE OF THE PARATHYROIDS ON THE CALCIUM CONCENTRATION OF MILK*

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The importance of the parathyroids in the regulation of over-all calcium metabolism led us to investigate the possibility that parathyroid hormone may influence the calcium-concentrating function of lactating mammary glands. Experiments on lactating rats did reveal changes in the composition of the milk 24 hours after removal of the parathyroid glands.

The concentration of calcium in the milk was increased markedly in spite of a greatly depressed serum calcium level. The milk appeared obviously thicker, and there was indeed an increase in the total solids. The increase in calcium content, however, was not entirely accounted for by the reduction in per cent water, as the calcium concentration, expressed as milligrams per gram of milk solids, was significantly higher after parathyroidectomy than in control sham-operated rats. Administration of parathyroid extract, immediately after parathyroidectomy, prevented the decrease in water content of the milk as well as the fall in serum calcium. The increase in calcium concentration of the milk was not so clearly prevented.

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* Abstract. Circumstances beyond the control of the authors and of The New York Academy of Sciences have prevented publication of the complete manuscript as presented at the conference upon which this monograph is based. The complete paper will be published elsewhere at a later date.

The investigation described in this paper was supported in part by research grants from the National Institute of Dental Research, Public Health Service (Grant No. D-173), Bethesda, Md.; the American Cancer Society, New York, N. Y.; the Charles H. Hood Dairy Foundation, Boston, Mass.; and the Milton Fund of Harvard University, Cambridge, Mass.

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Part III. Calium Complexing Agents

CHELATING AGENTS IN THE STUDY OF RENAL ABSORPTION OF ALKALINE EARTH CATIONS

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Georgetown University, Washington, D. C.

The kidney has been suggested as the locus of the body's homeostatic control of the alkaline earth cations, calcium and magnesium.[1] This contention is supported by the reasonable calculation that of the calcium filtered at the glomerulus, over 99 per cent must be actively resorbed by the kidney to maintain balance of this mineral in the body. Renal-clearance studies have affirmed the fact of this high level of kidney conservation of filtered calcium.[2] Minimal changes in this functional activity of the kidney could produce maximal and possibly fatal alterations in the body levels of calcium and magnesium. A basic problem in this field is an understanding of the mechanisms by which this highly integrated and mobile functional activity of the kidney is regulated in the living animal.

The techniques developed in the last few decades that have now become classic tools for the study of renal physiology encounter serious difficulties in application to this question of alkaline earth metabolism. Clearance studies, conducted as they must be in full awareness of the status of renal hemodynamics at the time of investigation, require a full, detailed, and explicit knowledge of the state of diffusable calcium in the plasma. The involved equilibrium between calcium bound as proteinate, in colloidal phosphate combination, in undissociated organic form, and in ionic state has defied rigorous understanding. The experimentally complicated direct measurement of the fraction of ultrafilterable calcium in the plasma has served as the basis of first calculation in this field, but still leaves a residuum of uncertainty as to whether all of this fraction is in a form amenable to the action of the kidney resorbing mechanisms. A further difficulty in renal studies by the application of calcium-loading techniques relates to the uncertainty of whether the experimental methodology has called forth compensatory alterations in kidney function. Despite these difficulties, the painstaking studies of many competent investigators have delineated the broad outlines of the renal handling of the alkaline earths. The purpose of this paper is to indicate some of the possibilities for a new view of this picture through the application of chelating compounds.

Chelation is the process of combination of a metal ion and an organic molecule with the formation of an intramolecular ring structure.[3] Naturally occurring chelates vary in complexity from the relatively simple structures, such as calcium citrate or the metal complexes of amino acids, to the complex planar chelate combination exemplified by the iron-porphyrin type molecule so essential in oxygen transport and intracellular oxidation. Many varities of synthetic organic molecular structures serve as ligands for cations. Of special interest for the subject under consideration is the group of polyamino polycarboxy acids exemplified by the structural types I, II, III and IV of FIGURE 1. For this sequence of compounds there is a direct relation between the metal-holding ability of the compounds and the number of intramolecular ring structures in a given compound. The very high order of stability of the metal complexes of ethylenediaminetetraacetic acid (EDTA) is sometimes ascribed to the formation of the postulated hexaring structure of type IV. For any given chelating compound there is a hierarchy of metal-binding ability for various cations. Metals higher in the scale of binding ability have the potential of displacing more weakly bound metals from chelate combination. This reaction is the basis of the successful clinical use of calcium disodium EDTA for the removal of toxic accumulations of metals, such as lead, from the body.[4-6]

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* From the thesis of G. E. Lindenblad submitted to Georgetown University, Washington, D. C., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

A second very significant cation competition is that of the hydrogen ion for bound metal. In this equilibrium relationship an increasing concentration of hydrogen ions will result in increasing dissociation of the bound metal. The quantitative relation between the concentration of hydrogen ions and the degree of binding of calcium by EDTA is illustrated in FIGURE 2. The decreasing concentration of calcium ions in equilibrium with EDTA is expressed on the ordinate as a numerically positive pM function by conversion to the log of the reciprocal of its concentration. This is an analogous relation to the description of the decreasing hydrogen-ion concentration of the abscissa expressed by the increasing pH value. It is evident that for calcium EDTA there is almost a millionfold gradient in concentration of calcium ions between pH 4 and pH 8.

Our interest in the possible application of calcium disodium EDTA as a tracer of renal mechanisms of calcium metabolism stemmed from the considerations outlined above, coupled with our own observations and those of other investi-

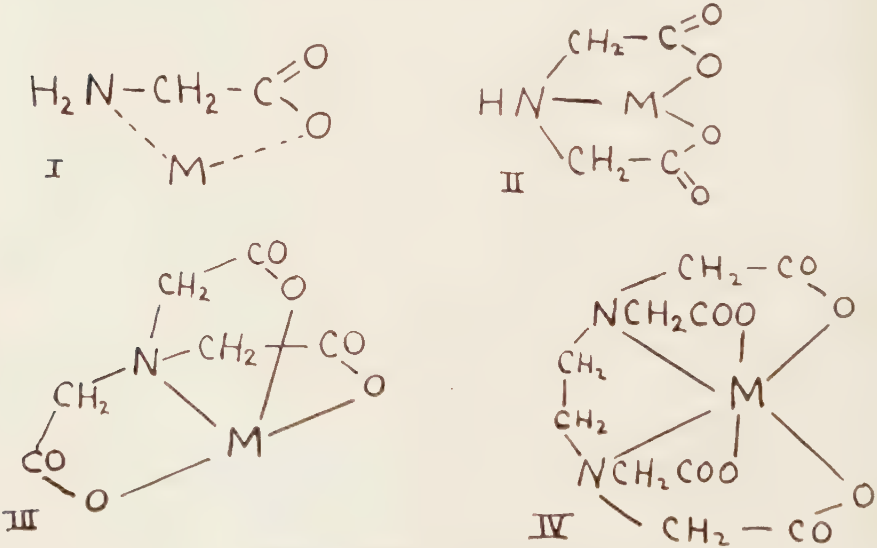

FIGURE 1. Structures of aminoacid chelates.

gators of the metabolism of this molecule.[7] Using radiocarbon tagged EDTA, Foreman and his colleagues have demonstrated in animals and humans that the organic half of the molecule is rapidly and completely excreted into the urine after parenteral administration.[8, 9] Available data on the urinary excretion of calcium after calcium disodium EDTA is somewhat less unambiguous. Spencer *et al.* in this country and several investigators abroad have reported on metabolic studies that show between 65 and 100 per cent recovery in the urine of calcium administered parenterally as calcium EDTA.[10-12] A second body of clinical evidence suggests that the injected chelated calcium is retained to a very much higher degree in hypocalcemic patients.[13, 14] If both groups of investigators are correct, there is the logical necessity that in the acutely hypocalcemic patient some homeostatic calcium mechanism has been evoked that abstracts calcium from an otherwise almost completely urinary excreted calcium EDTA. To test this possibility, rats were injected intraperitoneally with varying dos-

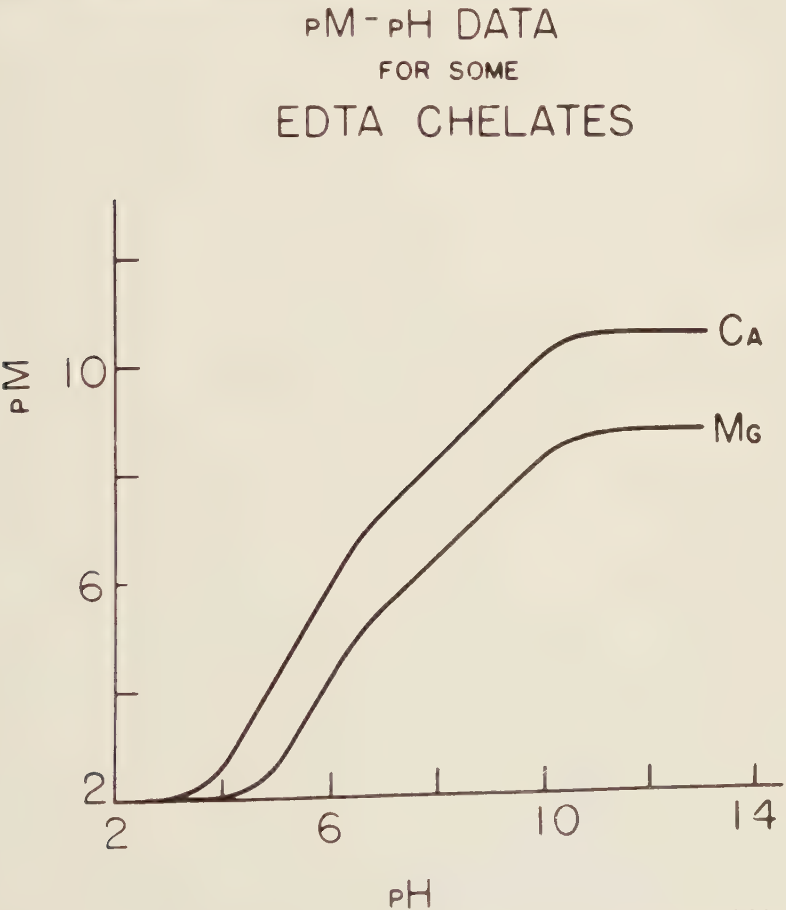

Figure 2. Relation of metal binding and hydrogen-ion concentration (after Martell and Calvin).

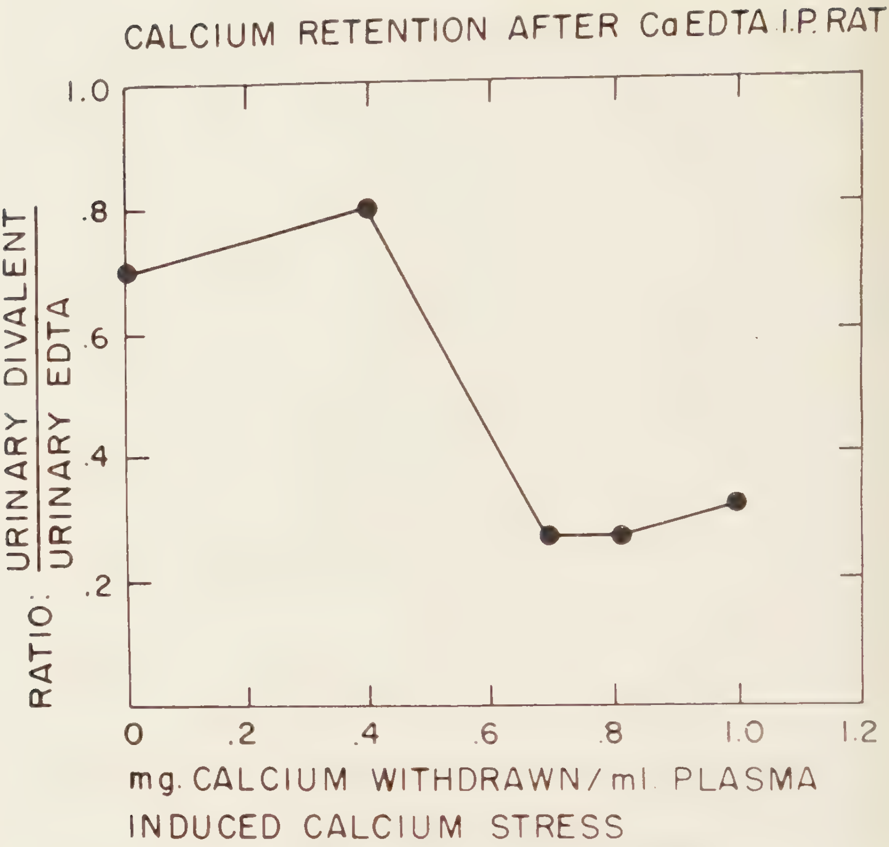

FIGURE 3. Alkaline earth metal ion retention in hypocalcemia.

ages of free EDTA in order to produce acute hypocalcemia.[15] At the same time, calcium EDTA was administered. The urinary output of EDTA was determined as described below, and the simultaneous output of the total of the urinary calcium and magnesium was measured. It is evident (FIGURE 3) that the urinary output of total alkaline-earth divalent ions following calcium EDTA normally, which is equivalent to about 75 per cent of the simultaneous urinary EDTA, drops to a minimal level of about 33 per cent when the animals are placed under the stimulus of acute hypocalcemic stress. Of interest is the fact that the nature of the dosage-response curve indicates a type of trigger mechanism with the evocation of a maximal physiological response.

The considerations above of the nature of the physical-chemical events able to bring about dissociation of the calcium from EDTA combination suggested the participation of pH change in the observed response. Further, it seemed reasonable to expect that such a change might occur in the renal system where both the regulation of pH and the resorption of mineral takes place. A group of animals was accordingly pretreated with effective levels of Diamox, a car-

TABLE 1

EXPERIMENTAL VARIATION OF URINARY DIVALENT ION/EDTA RATIO

Treatment	Ratio
Normal	0.75
Hypocalcemic stress	0.33
Hypocalcemia with Diamox	1.15
Hypocalcemia with sodium bicarbonate	0.85
Normal with ammonium chloride	0.63

bonic anhydrase inhibitor.[16] Repetition of calcium EDTA administration under conditions of hypocalcemic stress now resulted in complete excretion of the calcium with the EDTA (TABLE 1). Thus the known Diamox-inhibition of hydrogen-ion secretion in the tubule prevented the resorption of calcium from injected calcium EDTA, despite the concomitant stimulus of acute hypocalcemia. Confirmation of this interpretation of the findings was provided by the similar results obtained by means of sodium bicarbonate alkalosis. It was again evident that despite hypocalcemia, the alkaline kidney tubule inhibited resorption of calcium from calcium EDTA. It would be reasonable to expect from the above viewpoint that acidosis would promote the retention of calcium following calcium EDTA administration. While the results of attempted oral ammonium chloride acidosis have yielded suggestive evidence of such an effect, the results have not been dramatic. The normal urinary calcium EDTA ratio of 0.75 decreased approximately 15 per cent to 0.63 under these experimental conditions.

We feel that the sum of the results reported above justify two conclusions. First, it seems clear that in the areas of the tubule at which the hydrogen-ion alkali-metal exchange mechanism of resorption occur are also areas where alkaline earths may be reabsorbed. This, of course, does not exclude other kidney areas and other mechanisms for renal control of calcium and magnesium. Second, it appears that the stimulus of acute hypocalcemia provokes a renal response characterized by enhanced activity of the carbonic anhydrase base exchange mechanism.

Methods

Adult rats of both sexes of the Osborn-Mendel strain were used in this study. All the animals were maintained on the standard laboratory diet ad libitum. Water supply was also unrestricted. Urinary collections were made under metabolic conditions in which the feces were mechanically separated. Urines were made to volume and aliquots taken for analysis.

Total calcium and magnesium were determined on nitric acid wet-ashed aliquots of urine by titration with standard EDTA at pH 10 in ammonia buffer. The color change from wine-red to yellow-green of the Eriochrome Schwartz T indicator was used for the titration end point.[6] By waiting for at least 3 minutes to establish the permanency of the end point, the previously reported difficulties in this application of EDTA analysis in urine was obviated.[15] Recovery and control experiments showed an accuracy of ±5 per cent for the analysis.

For EDTA analysis, urine aliquots were decolorized by slurry with Nuchar C, followed by filtration. The clear water white filtrates were analysed for EDTA by conversion to the chromium chelate as described by Zak *et al.*[19]

Diamox in aqueous solution as the sodium salt was administered at the dosage of 8 mg. kg. by intraperitoneal injection. Calcium EDTA and EDTA were similarly injected in 12.5 per cent solution. Total dosage of the chelate was equivalent to approximately 100 ml. of 0.01 solution.

References

1. Smith, H. W. The Kidney. 1951. Oxford Univ. Press. New York, N. Y.
2. Neuman, W. F. & P. S. Chen, Jr. 1954. Metabolic interrelations. : 130. Josiah Macy, Jr. Foundation. Transactions of the Fifth Conference. New York, N. Y.
3. Martell, A. E. & M. Calvin. 1952. Chemistry of the Metal Chelate Compounds. Prentice Hall, New York, N. Y.
4. Bessman, S., H. Reid & M. Rubin. 1952. Med. Ann. Dist. Columbia. **21:** 312.
5. Rubin, M., S. Gignac, S. Bessman & E. Belknap. 1953. Science. **117:** 659.
6. Bessman, S., M. Rubin & S. Leikin. 1954. Pediatrics. **14:** 201.
7. Rubin, M. 1954. Metabolic Interrelations. : 355. Josiah Macy, Jr. Foundation. Trans. 5th Conf. New York, N. Y.
8. Foreman, H., M. Vier & M. Magee. 1953. J. Biol. Chem. **203:** 1045.
9. Foreman, H. & T. T. Trujillo. 1954. J. Lab. Clin. Med. **43:** 566.
10. Spencer, H., V. Vankinscott, J. Lewin & D. Lazlo. 1952. J. Clin. Invest. **31:** 1023.
11. Hofstetter, R. 1953. Schweiz. med. Wochschr. **83:** 611.
12. Petrides, P. & H. Wagner. 1953. Medizinische. : 821.
13. Hunzinger, W. & G. A. Ortelli. 1954. Schweiz. med. Wochschr. **84:** 1339.
14. Courvoisier, B., E. C. Bonard & E. Martin. 1954. Schweiz. med. Wochschr. **84:** 1249.
15. Popovici, A., C. Geshickter, A. Removsky & M. Rubin. 1950. Proc. Soc. Exptl. Biol. Med. **74:** 415.
16. Berliner, R. W., T. J. Kennedy, Jr. & J. Orloff. 1951. Am. J. Med. **11:** 274.
17. Friedman, H. S. & M. Rubin. 1955. J. Clin. Chem. **I:** 125.
18. Wilson, A. A. 1953. J. Comp. Pathol. Therap. **63:** 294.
19. Cherney, P. J., B. Crafts, H. H. Hagermoser, A. J. Boyle, R. Harbin & B. Zak. 1954. Div. Biol. Chem. 24th Meeting Am. Chem. Soc. Chicago, Ill.

THE SUPPRESSION OF URINARY CALCIUM AND MAGNESIUM BY ORAL SODIUM PHYTATE: A PRELIMINARY REPORT*

By Philip H. Henneman, Evelyn L. Carroll, and Fuller Albright
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Introduction

Studies by Mellanby,[1] McCance and Widdowson[2] and Nicolaysen and Njaa[3] over the past 25 years have demonstrated that diets rich in certain cereals and brown bread have a decalcifying action in rats, puppies, and humans. The chemical substance responsible for this action is phytic acid (inositol hexaphosphoric acid). These investigators have demonstrated that phytic acid interferes with calcium absorption and that this effect may be antagonized by increasing the calcium intake or by administering vitamin D. Interest in phytic acid apparently waned, however, when it became clear that the phytic acid content of the average British and Scandinavian diets, even in wartime, was not sufficiently large to interfere seriously with calcium absorption.

During the past 3 years we have demonstrated that certain patients with sarcoid may have hypercalcemia and hypercalcuria and that the mechanism of such hypercalcemia closely resembles that of vitamin D intoxication.[4] Accordingly, it was decided to study in such patients the effect of sodium phytate on the basis of its reputed blocking effect of the response to vitamin D.

Chemistry of Sodium Phytate

The theoretical structure of sodium phytate is shown in FIGURE 1. The exact physical chemical state of phytic acid or of sodium phytate is not known. A 15 per cent solution in water of sodium phytate† has a pH of 7.2 and is approximately 80 per cent pure sodium phytate, 15 per cent neutral sodium phosphate, and 5 per cent water.

Results

(1). *The effect of sodium phytate in a patient with sarcoid and hypercalcemia.* The subject of the first study was a 37-year-old man with sarcoid and recurrent renal stones in his sole remaining kidney. As shown in FIGURE 2, this study consisted of 24 days on a low-calcium intake and 72 days on a higher calcium intake achieved by the daily addition of 0.47 gm. calcium as calcium levulinate. After 12 days of control on the higher calcium intake, the patient received sodium phytate 8.8 gm. per day in divided doses for 18 days. Then followed 12 days off sodium phytate, 18 days on hydrocortisone 200 mg. per day, and a final 12-day control period. We are primarily interested in the calcium and magnesium balances shown at the bottom of the figure.

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* These studies were supported in part by grants from the American Cancer Society, New York, N. Y., on recommendation of the Committee on Growth of the National Research Council, Washington, D. C., the Rockefeller Foundation, New York, N. Y., and the Public Health Service, Department of Health, Education and Welfare, Bethesda, Md.

† Sodium phytate powder was supplied by Corn Products Refining Company, New York, N. Y. and, more recently, has been distributed by E. R. Squibb and Company, New Brunswick, N. J.

Note the extremely low fecal calcium excretion, averaging 8 mg. per day during the initial 24 control days. On this very low intake of calcium we should normally expect a fecal calcium level of approximately 200 mg. per day.[5] On the higher calcium intake, note that the patient went into positive calcium balance, due primarily to a smaller-than-normal fecal excretion of calcium. Thus this patient with sarcoid showed markedly increased absorption of calcium and hypercalcuria as a consequence.

When sodium phytate, 8.8 gm. per day in divided doses by mouth, was administered there was an immediate fall in urinary calcium to essentially nil values that continued throughout the 18 days of sodium phytate administration. On discontinuing sodium phytate, the urinary calcium promptly returned to control values. Note that the fecal calcium increased on sodium phytate to very nearly the same extent to which the urinary calcium decreased, so that the calcium balance was little altered by sodium phytate. On discontinuing sodium phytate, the fecal calcium returned to its previous level. The serum calcium was little changed, and the serum phosphorus rose during sodium phytate administration.

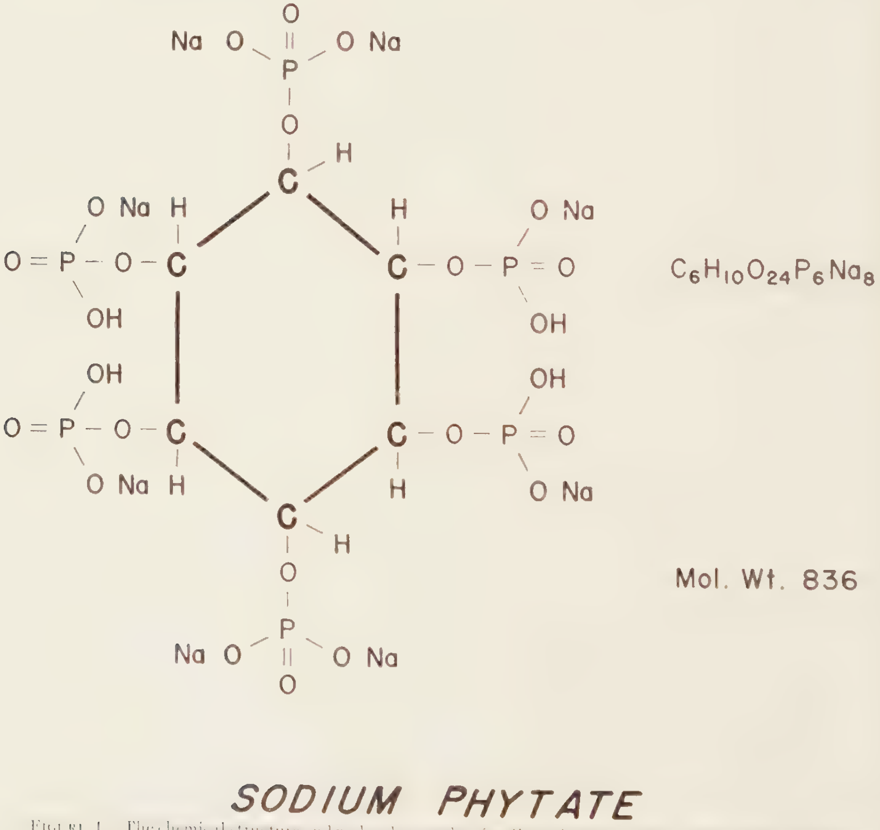

FIGURE 1. The chemical structure and molecular weight of sodium phytate. The presence of 8 sodium atoms per molecule of phytic acid is calculated from analysis of sodium phytate powder at pH 7.2.

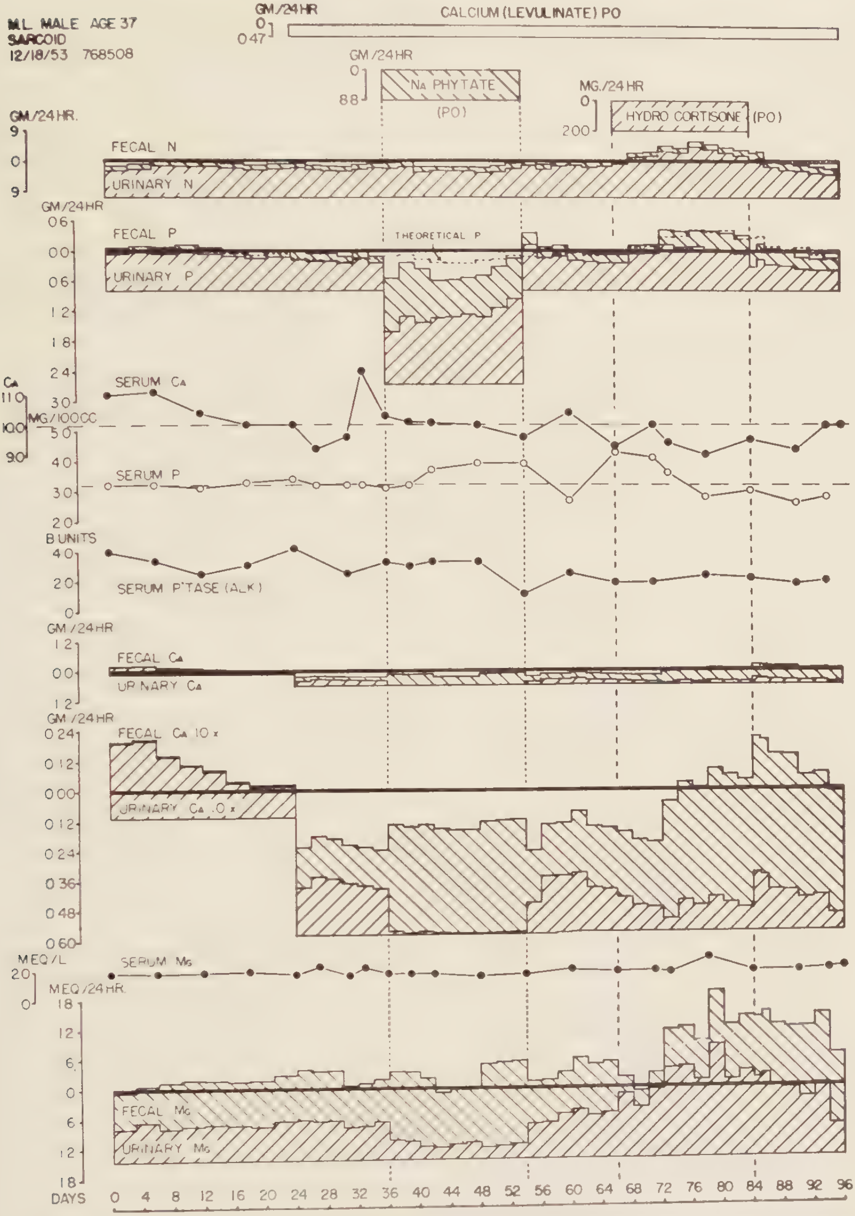

FIGURE 2. The effect of sodium phytate and cortisone acetate on calcium and magnesium balance in a patient with sarcoid and hypercalcemia. The intake of each substance is measured down from the heavy zero line; the fecal and urinary excretions are charted up from the intake line. The calcium balance is also charted at ×10 magnification for clarity. The scales for nitrogen, phosphorus, and calcium are chosen to reflect the ratio of the substances in protoplasm. The scale for magnesium is selected arbitrarily for clarity. The effect of cortisone is discussed elsewhere.[4]

During phytate administration, the urinary magnesium decreased to about half its control value. The fecal magnesium increased, and this increase was approximately the same as the decrease in urinary magnesium, resulting in no important net change in magnesium balance. There was no change in serum magnesium.

Thus sodium phytate produced marked increases in fecal calcium and fecal magnesium, and marked decreases in urinary calcium and magnesium in this patient with greater-than-normal calcium absorption.

(2). *The effect of sodium phytate in a patient with idiopathic hypercalcuria.*[6] This 48-day study extended over a period of 18 days before control (FIGURE 3), 12 days on sodium phytate, 6 days on neutral sodium phosphate, and 12 days after control.

On a low calcium intake of 130 mg. per day, this patient's fecal calcium averaged 160 mg. per day, or about half the usual value on this intake. His urinary calcium averaged 150 mg. per day, or more than twice the normal urinary calcium excretion for this level of calcium intake.[5] The administration of sodium phytate resulted in a decrease of the urinary calcium to a normal level of 60 mg. per day. The fecal calcium increased to 200 mg. per day. On stopping sodium phytate, the urinary and fecal calcium returned to control values. The serum phosphorus was somewhat elevated by sodium phytate administration. The urinary magnesium decreased and the fecal magnesium increased to a similar extent on sodium phytate. There was little change in serum calcium or magnesium.

Since approximately half of the phosphorus of the administered sodium phytate is hydrolyzed and absorbed, this patient was given, next, a neutral phosphate solution to identify the effect of the absorption of added inorganic phosphorus on calcium and magnesium metabolism. The urinary phosphorus increased considerably on 3.5 gm. neutral phosphate solution, though not quite to the same extent as it had on 8.8 gm. sodium phytate daily. The urinary calcium was slightly decreased, the fecal calcium was unchanged, and magnesium metabolism was probably unaltered by this amount of neutral inorganic phosphate.

Thus in this patient, whose calcium absorption was only slightly greater than normal, the administration of sodium phytate reduced to normal the urinary calcium and increased to normal the fecal calcium. This resulted in a greater degree of negative calcium balance, but this degree of negative calcium balance would be expected in a normal subject on this very low-calcium intake.[5] It would appear that some of the urinary calcium-decreasing action of sodium phytate was due to the absorption of large amounts of inorganic phosphorus, since neutral sodium phosphate solution slightly reduced the urinary calcium excretion in this patient. This effect, however, was slight in degree as compared with the much larger effect of sodium phytate.

The Long-Term Effects of Sodium Phytate Administration

Walker and co-workers[7] have indicated a waning of the effect of sodium phytate on long-continued administration and have suggested that there is

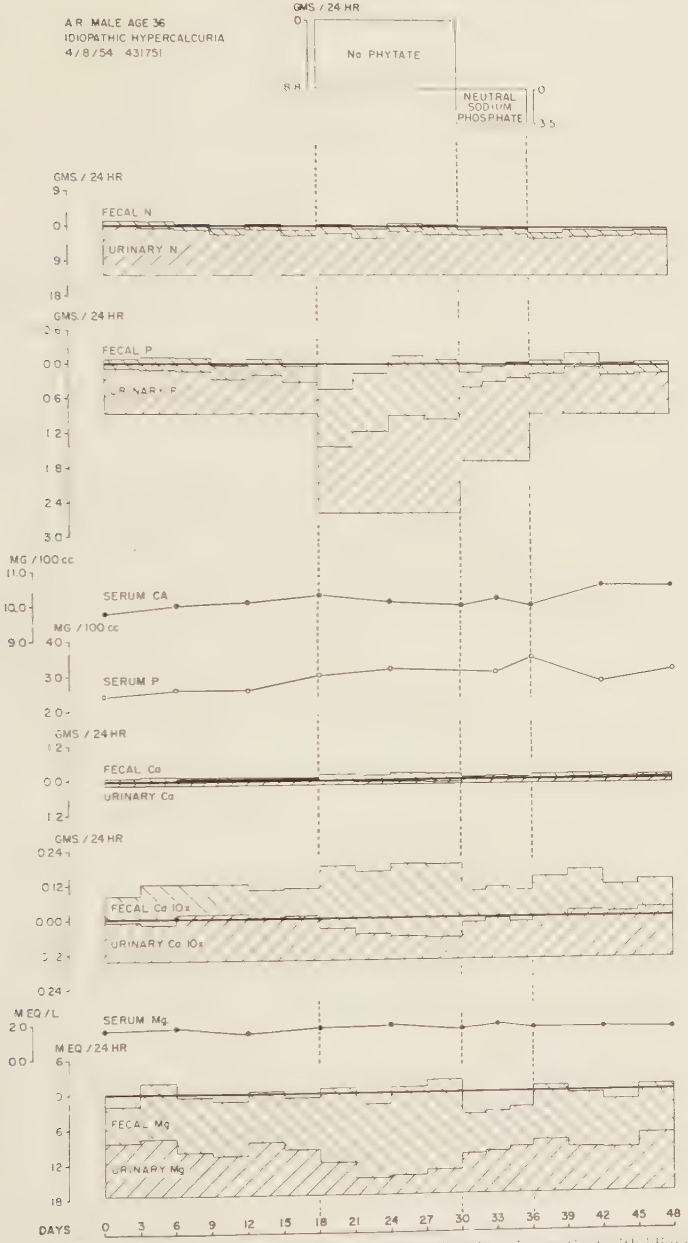

FIGURE 3. The effect of sodium phytate and of neutral sodium phosphate in a patient with idiopathic hypercalcuria. The subject of this experiment was a 36 year old white male. By idiopathic hypercalcuria we refer to the condition of a group of men with renal stones, normal serum calcium, slightly lower than normal serum phosphorus, and slightly higher than normal urinary calcium. The exact mechanism of this syndrome is not known.

adaptation to the specific effects of sodium phytate. FIGURE 4 illustrates the effect of long-continued sodium phytate administration to the subject of the first balance study. Note that his urinary calcium remained low; indeed, most values were below 50 mg. per day throughout the 13 months of sodium phytate administration. The patient was stone free and infection-free during these 13 months. After discontinuing sodium phytate, the urinary calcium returned to, and persisted at, extremely high values, indicating that the abnormality of calcium metabolism was still present. Certainly there is no evidence of loss of effect of sodium phytate over the 13 months of this study, and the patient demonstrated no ill effects from the prolonged sodium phytate administration.

FIGURE 5 illustrates the effect of long-continued sodium phytate administration on the urinary calcium excretion of the subject of the second balance study. The urinary calcium excretion of this subject after prolonged phytate administration was higher and more irregular than that of the previous subject, but was lower than his own pretreatment values. Furthermore, there was no evidence of the waning of the effect of sodium phytate during prolonged administration. This patient shows no ill effects of prolonged phytate administration, and X rays demonstrate no recurrence of renal stones.

Toxic Effects of Sodium Phytate Administration

We have administered sodium phytate during balance studies to 5 subjects and to approximately 36 outpatients for periods ranging from a few days to 18

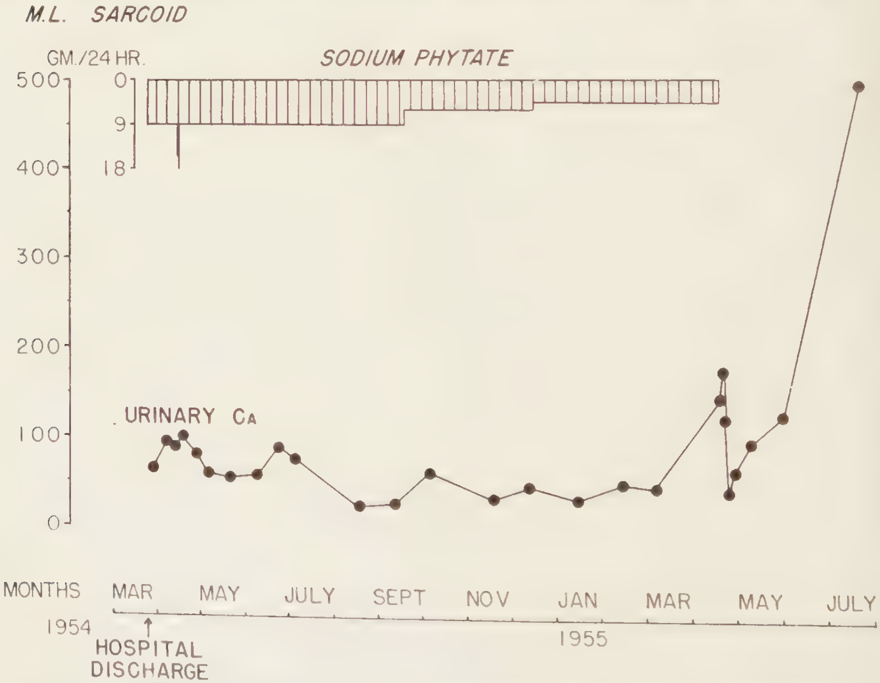

FIGURE 4. The effect of prolonged sodium phytate administration to M. L., a patient with sarcoid and hypercalcemia. See FIGURE 1 for urinary calcium excretion prior to treatment. The scale for urinary calcium is in mg. per 24 hours.

months. In all subjects there has been some reduction of the urinary calcium and magnesium excretion, and this effect has not waned with prolonged administration of phytate. The main toxic effect noted to date is a diarrhea in all subjects that is painless and that tends to ameliorate after 3 to 6 weeks of phytate administration. We have not noted any effect of prolonged phytate administration on the blood or other organs of the body.

Discussion and Summary

The evidence from these 2 balance studies indicates that sodium phytate in large doses may markedly decrease the urinary excretion of calcium and magnesium. One third to one half of the administered phytate apparently is not absorbed or hydrolyzed and probably forms insoluble and unabsorbable complexes with calcium and magnesium. As a consequence of decreased absorption, the urinary excretion of calcium and magnesium decreases. The degree of depression of the urinary calcium and magnesium excretion depends in part

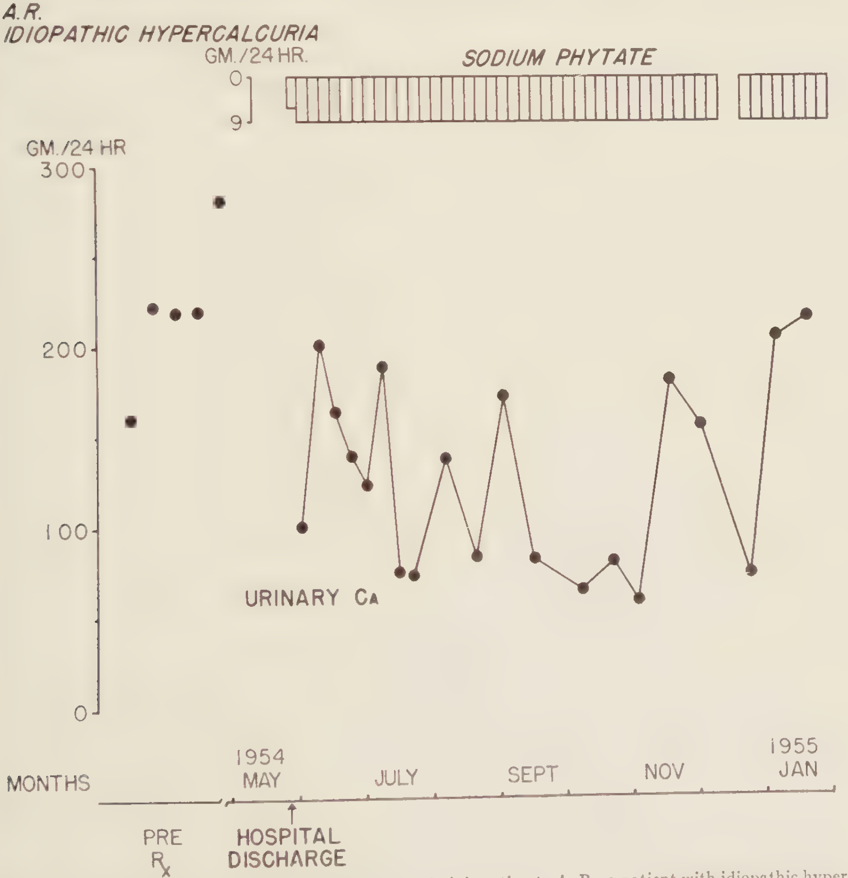

FIGURE 5. The effect of prolonged sodium phytate administration to A. R., a patient with idiopathic hypercalcuria. The scale for urinary calcium is in mg. per 24 hours. The patient was on a free diet without milk or cheese.

on the phytate dosage and in part on the magnitude of calcium and magnesium absorption. Thus the effect of phytate on urinary calcium excretion is greatest in vitamin D poisoning and sarcoid, where calcium absorption is markedly increased.

One half to two thirds of the phytate phosphorus is hydrolyzed and absorbed, increasing the serum phosphorus level. By mass action, this tendency probably favors the deposition of calcium and phosphorus in bone, reduces the serum calcium, and results in a decrease in urinary calcium excretion. Such an effect of phytate is most apparent in circumstances of increased urinary phosphorus excretion, such as hyperparathyroidism and excessive vitamin D effect. The increased absorption of phosphorus probably does not alter magnesium metabolism.

It is of considerable theoretical interest that 9.0 gm. per day of sodium phytate does not materially alter calcium balance, which suggests that the absorption of dietary calcium is blocked, whereas there is no interference with the reabsorption of calcium secreted into the intestine through the bile and other intestinal juices. It has been our experience that a similar situation exists with phosphorus metabolism. Aluminum gels will block the absorption of dietary phosphorus, but even in very large doses will not produce negative phosphorus balance, suggesting that the phosphorus of the intestinal juices may be in a different form from that of the diet.

Sodium phytate appears to be a safe therapeutic agent in adults and of value in the treatment of vitamin D poisoning, sarcoid with hypercalcemia, and certain renal stone syndromes. Its value in other hypercalcemias and hypercalcurias remains to be investigated. In growing children who must remain in positive calcium balance, sodium phytate in large doses may prove rachitogenic.

References

1a. Mellanby, E. 1925. Med. Research Council Brit. Spec. Rep. Ser. No. 93.
1b. Mellanby, E. 1949. The rickets-producing and anti calcifying action of phytate. J. Physiol. **109:** 488.
2. McCance, R. A. & E. M. Widdowson. 1942. Mineral metabolism of healthy adults on white and brown bread dietaries. J. Physiol. **101:** 44.
3. Nicolaysen, R. & B. R. Njaa. 1951. The effect of phytic acid on the absorption of calcium in rats, pigs and men. Acta Physiol. Scand. **22:** 246.
4a. Henneman, P. H., E. L. Carroll & E. F. Dempsey. 1954. The mechanism responsible for hypercalcuria in sarcoid. J. Clin. Invest. **33:** 246.
4b. Henneman, P. H., E. L. Carroll, E. F. Dempsey & F. Albright. The cause of hypercalcuria in sarcoid and its treatment with cortisone and sodium phytate. To be published.
5. Bauer, W., F. Albright & J. C. Aub. 1929. Studies of calcium and phosphorus metabolism. II. The calcium excretion of normal individuals on a low calcium diet, also, data on a case of pregnancy. J. Clin. Invest. **7:** 75.
6. Albright, F., P. H. Henneman, P. B. Benedict & A. P. Forbes. 1953. Idiopathic hypercalcuria (a preliminary report). Proc. Roy. Soc. Med. **46:** 1077.
7. Walker, A. R. P., F. W. Fox & J. T. Irving. 1948. Studies in human mineral metabolism. 1. The effect of bread rich in phytate phosphorus on the metabolism of certain mineral salts with special reference to calcium. Biochem. J. (London). **42:** 452.

OSTEOMALACIA IN NORTH CHINA: ITS RELATIONSHIP TO PREGNANCY AND LACTATION

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For years, Rudolf Virchow's concept that rickets and osteomalacia were two different diseases was generally accepted. As early as 1885, however, Pommer[1] insisted that the two diseases were identical. Later Schmorl,[2] Looser,[3] and others collected histologic evidence that confirmed Pommer's views. After the discovery of vitamin D, the influence of a deficient vitamin D intake upon the pathogenesis of both rickets and osteomalacia became evident. Only then was the unitary concept of the two diseases generally accepted. Everyone is now in agreement that osteomalacia of the adult[4] is the same disease as rickets of the child and adolescent.

There are different conditions that may lead to avitaminosis D. This deficiency may be the result either of an insufficient intake of vitamin D combined with lack of exposure to sunlight or it may be caused by loss of vitamin D. Losses of vitamin D occur in the course of fatty diarrhea, whether this is caused by sprue, celiac disease, chronic jaundice, chronic pancreatitis, malignant or tuberculous infiltration of the mesenteric lymph nodes, or Whipple's disease.[5] In these cases, not only the absorption of fats but also of fat-soluble vitamin D suffers, and typical osteomalacic bone lesions become manifest despite a satisfactory oral intake of vitamin D.

Gradually it has become understood that rickets and osteomalacia may also occur in patients whose intake and intestinal absorption of vitamin D are apparently sufficient. In such cases a tubular dysfunction exists that leads to a decreased reabsorption of phosphates in the renal tubules.[6, 7] This remarkable condition causes intense hyperphosphaturia and, due to the loss of phosphates, insufficient deposition of calcium phosphate in the bone matrix ensues. The osteomalacia caused by tubular dysfunction does not respond to current therapeutic doses of vitamin D. This explains why this skeletal anomaly is often designated as "vitamin D-resistant rickets," a name that is not completely correct because the disease can be favorably influenced by tremendously large doses of vitamin D (that is, 500,000 units).

Since this report is limited to the influence of pregnancy and lactation upon calcium metabolism, only the disturbances due to insufficient vitamin D intake combined with lack of exposure to sunlight will be discussed.

Most of the skeletal and metabolic changes in avitaminosis depend upon the fact that only in the presence of vitamin D can calcium be absorbed from the intestine. Thus avitaminosis D leads, via insufficient calcium absorption from the intestine, to insufficient calcification of bone matrix. Modern experimental research indicates that, apart from its action upon intestinal calcium absorption: (1) vitamin D may also directly influence calcification of cartilage; (2) vitamin D directly influences the function of the renal tubules and thereby modifies urinary phosphorus and calcium excretion; and (3) very large doses of vitamin D may even cause resorption of bone.

Biochemical Signs

Since the absorption of calcium from the intestinal canal is reduced to a minimum in the absence of a sufficient amount of vitamin D, the fecal calcium excretion is increased in avitaminosis D, and the calcium content of the urine necessarily decreases. In fact, in most cases of avitaminosis D the urinary calcium drops to traces. When in avitaminosis D the phosphorus intake is adequate, the urinary phosphate excretion remains within normal limits.[8] This makes it highly probable that in avitaminosis D an efficient intestinal absorption of phosphorus exists. When in avitaminosis D the calcium intake is increased to very high values, however, the nonabsorbed intestinal calcium forms large quantities of insoluble calcium phosphates that are eliminated in the stools. Under these circumstances the excretion of phosphorus in the stools significantly increases and the phosphaturia dwindles to traces.

The influence of vitamin D upon the function of the kidneys mentioned above may lead to changes in calcium and phosphorus excretion in the urine, independent of the calcium and phosphorus content of the plasma. This probably explains why the first sign of osteomalacia often consists of the absence of calcium in the urine of patients even in cases where the serum calcium varies between 9 or 9.5 mg. per 100 cc., that is within the lower limits of normal.

In later stages, avitaminosis D leads to a decrease of serum calcium and also of serum phosphorus. It is remarkable that, notwithstanding the satisfactory intestinal absorption of phosphates from the intestine and the ensuing normal phosphaturia, the serum phosphate in avitaminosis D is usually low. Modern clinicians have explained this paradox as the result of the moderate hyperplasia of the parathyroids that always develops in osteomalacia as it does in all other conditions with long-standing hypocalcemia. This hyperplasia of the parathyroids may well cause an increased secretion of parathyroid hormone. The hyperparathyroidism would cause hyperphosphaturia with ensuing hypophosphatemia, which would explain the hypophosphatemia present in rickets and osteomalacia. At the same time, the weakening of the bones due to impaired calcification results in an increased activity of the osteoblasts. This in turn causes an increase of the alkaline phosphatase of the serum. The diagnosis of avitaminosis D is proved when administration of vitamin D causes, within a week, an improvement of the calcium absorption from the intestine, as shown by an increase in the urinary calcium excretion and a decrease in the calcium content of the stools. Roentgenologic improvement of the calcium content of the bones becomes visible much later.

Administration of vitamin D to patients with avitaminosis D also causes an increase of the serum phosphorus. In part, this may be the result of improved calcium absorption from the stools, under the influence of vitamin D administration. When more calcium is absorbed from the intestine, less calcium phosphates are formed in the gut. This leads to increased phosphate absorption. Other investigators are of the opinion that the increase of the serum phosphorus may be due to a change of the function of the parathyroids. The rise in the calcium content of the serum of the osteomalacic patient that follows the ingestion of vitamin D results in a decreased function of the hyperplastic parathyroids. This leads to decreased excretion of phosphate in the urine and thereby to an increase of the phosphate of the serum.

Histology and Pathologic Physiology

Two different processes must be distinguished in the formation of bone. First, the osteoblasts lay down a matrix for the bone formation, consisting of a tissue rich in collagen and mucopolysaccharides. In a second stage, this matrix is calcified. Any disease in which faulty calcification of a normal matrix occurs must be designated as rickets or osteomalacia. In these two diseases, osteoid zones—that is, uncalcified matrix—are found around the bone trabeculae. Avitaminosis D is the cause of the rickets found in small children. This condition was frequent in Europe and the United States before the importance of sunshine and vitamin D was recognized. This deficiency state is also responsible for the different forms of rickets and osteomalacia that are endemic in large areas of the Orient.

Whereas broad osteoid seams surrounding the bone trabeculae are found in both rickets and osteomalacia only in rickets can characteristic changes at the cartilage-shaft junctions of bone be elicited. In children and young individuals the epiphyseal disks are open, and endochondral bone formation is active. In rickets, accordingly, faulty calcification is found in the provisional calcification zone of the epiphyseal disk, as well as in the osteoid zones. These changes of the epiphyseal calcification are visualized on the roentgenograms as irregular and broad epiphyseal disks. Since in an adult the epiphyseal disks are ossified, in osteomalacia the main changes can occur only in cortex and cancellous bone.

The identity of rickets and osteomalacia is practically proved by the proliferation of the cartilage that is found in the costochondral junctions of the ribs of the osteomalacic patient. The costochondral junction is the only place in the adult organism where a cartilage-bone junction persists, and here the same alterations can be visualized as are found in the cartilage-shaft junctions of children with rickets (Schmorl,[2] Fromme[9]).

At roentgen examination, the abnormally low calcium content of the bones is evident both in rickets and in osteomalacia. Under normal conditions, daily wear and tear takes place in the skeleton. A certain amount of bone is resorbed; this is then balanced by reconstruction of new bone. Since in rickets and osteomalacia this regenerative ossification is inhibited, the calcium content of the bones of such patients must diminish progressively. The insufficient calcification of the skeleton is less extensive in rickets than in osteomalacia. This difference can be explained by the fact that osteoclastic bone resorption is nonexistent during the first 8 months of life and, in childhood, remains less active than in the adult.

The presence of broad osteoid zones surrounding the bone trabeculae confirms the diagnosis of rickets and osteomalacia. It seems contradictory to study these osteoid zones in histologic bone sections that have been prepared by prolonged decalcification in strong mineral acid. The possibility that structural changes caused by exposure to strong acids could lead to confusing histologic pictures—that is, to pseudo-osteoid zones—must certainly be considered. It is therefore preferable to search for the presence of osteoid zones in fresh preparations of cancellous bone. Cancellous bone obtained at biopsy or autopsy is ground in a mortar. Tiny pieces of bone are mounted in 5 per cent silver nitrate solution. The phosphates of the bone trabeculae turn black by the formation of silver phosphate and, under low-power magnification the unstained broad osteoid zones of rachitic or osteomalacic bone trabeculae can easily be recognized.

Different Forms of Avitaminosis D

Avitaminosis D leads to fetal rickets in newborn children, to infantile rickets in infants below the age of 3 years, to late rickets in children older than 3 to 4 years and in adolescents, and to osteomalacia in adults. In late rickets, the same fundamental changes are found as in early rickets and osteomalacia. The epiphyseal disks remain open and increase in size. At the same time, osteoid seams and insufficient calcification of the bone trabeculae are prevalent. Roentgenologically, lack of calcification is usually more extensive than in infantile rickets, although perhaps less marked than in osteomalacia.

Osteomalacia is still endemic in the northern parts of India, Japan, and many provinces of north China. In these regions, the food is very poor in calcium, not high in phosphorus, and very low in vitamin D. Under these circumstances, avitaminosis D can be prevented only by exposure to sunshine. In China, milk and butter are unknown as dietary staples, and eggs, liver, sea food, meat, fowl, and other animal products can be eaten only by the better-situated classes. The main source of calories for the poorer classes of north China consists of cereals, mostly millet, kaoliang (broom corn), and maize, eaten with varying amounts of vegetables. The somewhat better-situated people use wheat and soybean flour, with considerable amounts of fresh vegetables.[10] Since vitamin D is present only in animal food, the vitamin D intake in north China is practically nil. Furthermore, this diet is poor in calcium, due to the absence of milk and dairy products. The average daily calcium intake of the poor Chinese in north China did not exceed 334 mg., and often varied between 200 to 250 mg.

In most patients in Peiping no clear-cut clinical signs of this disease could be found. The unfavorable influence of the low-vitamin D and low-calcium intake was in most cases neutralized by the sunny character of the north Chinese climate. The average number of hours of sunshine in north China equals that found in Hawaii. As long as the population works out-of-doors, therefore, a sufficient amount of vitamin D is produced in the skin, and no avitaminosis D can develop. As soon as an individual is confined indoors, however, signs of active avitaminosis D may develop. This pattern is characteristic of most of the cases of rickets and osteomalacia endemic in north China.

Maxwell[11, 12] has done fundamental work in this field for China. He and his associates Miles and Feng, in 1925, confirmed the observation that in osteomalacia the calcium balance is always negative.[13] In addition, they proved that this negative calcium balance was not due to a low-calcium intake, but that a deficient intake of a fat-soluble vitamin was much more important for the pathogenesis of osteomalacia. Calcium was retained by osteomalacic patients under the influence of cod liver oil. After administration of the latter preparation, clinical improvement resulted even when the calcium intake in the diet was not increased. On the other hand, an increased calcium intake without addition of the fat-soluble vitamin fraction did not improve the clinical condition. A long series of experiments in the metabolic ward of the Peiping Union Medical College, Peiping, China, confirmed and elaborated their original results.[14-19]

Influence of Pregnancy and Lactation on Calcium and Phosphorus Metabolism of Women with Avitaminosis D

Osteomalacia is much more frequent among women than men, although both sexes follow the vegetarian diet previously mentioned. Household duties keep the women indoors, however, so that their exposure to sunshine is minimal, while the men, particularly of the poorer class, work in the open. Moreover, pregnancy and lactation constitute a drain on the calcium content of the female skeleton.

Pregnancy leads to considerable losses of calcium, phosphorus, and vitamin D from the maternal organism. This is easily proved by the following figures. The skeleton of the newborn child contains between 21 and 23 gm. of calcium, together with 14 gm. of phosphorus. Occasionally, even a calcium content of 30 gm. has been found. At the same time, significant amounts of vitamin D are deposited within the body of the fetus.

Two thirds of the total amount of calcium of the fetal skeleton are deposited during the last 3 months of pregnancy. The placenta also contains a considerable quantity of calcium. Thus the calcium balance of the maternal organism must be positive during pregnancy, otherwise the calcium required for the formation of the skeleton of the fetus will be lost by the maternal skeleton. The pregnant woman must retain 50 mg. of calcium every day between the third and the fifth lunar month of pregnancy, 120 mg. between the seventh and ninth lunar month, and 450 mg. during the 10th lunar month. Other investigators believe that a minimum of 200 mg. of calcium and 100 mg. of phosphorus must be retained daily during the last three lunar months of pregnancy.

The calcium requirements necessary for a satisfactory calcium balance are not the same in all countries of the world. It is well known that a population such as the Chinese, accustomed to a low-calcium intake, requires less calcium to remain in calcium balance than, for instance, the inhabitants of North America, whose calcium intake is surprisingly high. This also holds true for the calcium balance during preganancy. A pregnant Chinese woman provided with a satisfactory amount of vitamin D can maintain her calcium balance with a daily calcium intake of 500 mg. This would be satisfactory under normal conditions. Since, however, the calcium balance must be strongly positive during pregnancy, the pregnant Chinese woman requires a daily calcium intake of 1000 mg. All this holds true only if sufficient vitamin D is provided. In avitaminosis D the calcium balance of the pregnant Chinese woman remains strongly negative even if the daily calcium intake is increased to 1275 mg.

Lactation presents a much heavier burden than does pregnancy on the calcium metabolism of the mother. The calcium content of mother's milk varies from 25 to 30 mg. per 100 cc. Thus during the first months of lactation, when 1000 to 1200 cc. of milk are produced each day, the mother loses between 300 and 400 mg. of calcium daily. Coupled with this is the loss of relatively large amounts of vitamin D present in the mother's milk. This is of major importance in nearly all Oriental countries, where lactation is continued for an average of 2 years. It must be added that in the later months of lactation, losses of calcium and vitamin D are curtailed by the progressive decrease in the daily quantity of milk produced. The lactating Chinese mother who receives satisfactory amounts of vitamin D can remain in calcium balance if the calcium intake varies between 1 and 2 gm. Since the calcium balance of any woman with avitaminosis D is negative, even if she is not pregnant or not lactating, pregnancy (and, to an even greater extent, lactation) must have a devastating effect upon the skeleton of the mother whose vitamin D intake is unsatisfactory.

As far as the newborn is concerned, the quantity of calcium offered to the child is always sufficient, even if the lactating mother suffers from avitaminosis D, since the latter condition does not affect the calcium content of the milk. The calcium metabolism of the breast-fed infant therefore depends completely upon the vitamin D content of the mother's milk.

The amount of the calcium present in the skeleton at birth varies around 0.7 per cent of the total body weight. This percentage climbs gradually until it reaches 1.6 per cent in the adult. In order to keep the calcium content of the skeleton of the growing infant around 1 per cent, it is necessary that the breast-fed child should retain 170 to 310 mg. of calcium daily.

As long as the mother receives a sufficient amount of vitamin D, the milk contains adequate quantities of this vitamin, and the calcium balance of the infant is strongly positive. Under these circumstances the child retains the necessary 76 to 82 per cent of the 300 to 350 mg. of calcium provided by the milk. Then the calcium excreted by the child is partly eliminated via the stools, partly via the urine.

When the mother's milk does not contain vitamin D, the infant suffers from avitaminosis D. In this case all the calcium excreted is eliminated via the stools, and calcium is no longer present in the urine. Even under these circumstances the calcium balance of the infant with avitaminosis D remains positive. Such children may still retain 60 to 140 mg. of calcium daily, an amount that, is nevertheless far below the 200 to 300 mg. the growing infant requires daily. Rickets must necessarily ensue or more frequently the fetal rickets with with the infant was born are not cured.

Why is the calcium balance of the avitaminotic breast-fed infant still positive, albeit not sufficiently, whereas the older rachitic child or the osteomalacic adult has a negative calcium balance?

It has already been mentioned that in the normal skeleton of the adult a certain amount of daily wear and tear takes place. Every day, part of the bone substance is resorbed and an identical quantity of bone is newly formed. In contrast, during the first 8 months of postuterine life no osteoclastic function exists, and no daily wear and tear of the skeleton occurs. During this early growing stage, only construction—no destruction—of bone substance takes place. Thus in the adult with osteomalacia, the calcium that is freed from the bones causes the calcium balance to remain negative. In infants with fetal rickets who are fed by osteomalacic mothers, no calcium is liberated from the skeleton and no negative calcium balance results. The balance remains positive, but nevertheless not enough calcium is retained to ossify all the rapidly proliferating bone matrix.

Discussion

These biochemical data explain why osteomalacia is frequent among the women in north China who stay indoors. There is no osteomalacia among the peasant women of the poorest class, however, for they work in the fields all day.

Comparable conditions prevail in other parts of the world. In Kashmir, India, for instance, the poorest women who work as boatwomen never have osteomalacia. Their nutrition is very deficient, but they work the whole day in the sunshine. Osteomalacia, however, is frequent among the women of the well-to-do classes who strictly observe purdah and stay indoors for the greater part of the day.[20, 21]

It can now be understood why careful probing into the history of osteomalacic women reveals that the onset of bone pains starts during lactation and not during pregnancy. Only the heavy losses of calcium and vitamin D that occur during lactation are capable of initiating the disease. Once osteomalacia has developed, the moderate losses of calcium and vitamin D during pregnancy are sufficient to cause a relapse or a deterioration of the disease. This process explains why, in areas where osteomalacia is frequent, children are born with fetal rickets. Due to the negative calcium balance of the mother, the maternal organism cannot provide enough calcium for the normal fetal calcification. It is also possible that, in addition to the absence of vitamin D, certain enzymatic processes necessary for the calcification of cartilage are changed. Thus the child is born with fetal rickets.

Infantile rickets in north China is relatively benign,[22] compared to the form of the disease that was commonly observed in the West. Rickets and complicating tetany occur in China in the first few months of postuterine life whereas, in the West, this disease was only rarely observed before the age of 6 months.[23] The mother's milk in the West could prevent rickets during breast feeding for, thanks to the generous amounts of vitamin D in the maternal diet, the mother's milk was rich in vitamin D. Rickets therefore usually occurred after breast feeding had been stopped, that is, after the sixth month of life, when the child's diet was limited to boiled or sterilized cow's milk. The milk of osteomalacic mothers, however, is poor in vitamin D and, for this reason, breast feeding is not as good a protection against rickets and tetany in Peiping as it is in New York. Chu and Sung[23] have reported that of 176 cases of rickets observed in the pediatric ward of the Peiping Union Medical College, 60 per cent of the infants had been breast fed—a vivid illustration of the low vitamin D content of mothers' milk there. This again accentuates the fact that rickets occurs even if the child's calcium intake is satisfactory. The calcium content of the milk of the osteomalacic mother is normal. This also serves to explain the remarkable frequency of tetany during the first 2 or 3 months of life in Peiping, as compared to the infrequency of tetany at this age in the West. In north China, however, infantile rickets disappears before the patient is 6 months old. As soon as the child makes efforts to crawl around it is allowed to get acquainted with the outer world. Scantily clad, it is placed in the open court of the house, and the sunshine cures the rickets before the child has reached the age of 6 months.

Late rickets in adolescents develops only under special circumstances; for example, in orphan girls educated in large institutions. The boys usually go about in the streets, while the girls are kept indoors, out of the sunshine, by the many household chores. It is possible that the menstrual flow withdraws some calcium each month.

Summary

The conditions found in north China and other parts of the Orient, can be summarized as follows:

(1) Fetal rickets may occur during the development of the fetus *in utero*, if the mother suffers from avitaminosis D.

(2) Rickets and tetany not infrequently occur even in the first few months of life, because the vitamin D content of the osteomalacic mother's milk is extremely low.

(3) In north China, severe infantile rickets is a rarity after the child is 6 months old. At this age, the infants already crawl and play out of doors in the sunny north Chinese climate.

(4) Late rickets is occasionally observed in adolescents, particularly girls, living in orphanages under abnormal conditions.

(5) After marriage, osteomalacia becomes frequent in women, for their household duties limit their exposure to sunshine. Furthermore, the losses of calcium from the skeleton during pregnancy and lactation become a major factor, due to the poor calcium absorption from the intestine that ensues from the extremely low intake of vitamin D in the food.

(6) High calcium content of the diet alone does not protect the skeleton.[24] Whether the calcium intake is high or low, no calcium is absorbed from the intestine in the absence of vitamin D. On the other hand, the low-calcium intake of the Chinese population in general is sufficient to maintain health as long as sufficient vitamin D is available. During pregnancy and lactation, however, the Chinese mother requires extra calcium and vitamin D in order to prevent osteomalacia of the mother and rickets of the newborn.

(7) In contrast to the findings in north China, the infantile rickets so frequent in our part of the world until about 1930 started only when the infant was about 6 months old. The child during its intrauterine development had received so much vitamin D from its well-fed mother that, at birth, its vitamin D stores were well filled. The child lived on these stores during the first months of

extrauterine life, even if it was fed with cow's milk, which at that time was not irradiated and therefore practically free of vitamin D.

After 6 months, the infantile stores of vitamin D were exhausted and rickets developed. This persisted until the child received a more liberal diet and was allowed out in the sun. Thus when the child was about 2 years old, the rickets spontaneously subsided.

Addendum

Since in the Western part of the world fetal rickets has not occurred for many decades, an occasional author still doubts whether the syndrome designated as fetal rickets actually exists. This may be the justification for a report of the observations made on a baby born to a mother who had shown signs of osteomalacia for ten years.[25] She had had many pregnancies during these years, in the course of which she had always suffered from severe bone pains and convulsions. For the last 6 months of this pregnancy she had been hardly able to walk.

The child was born about 1 month before term. A certain amount of artificial feeding had been necessary to supplement the breast feeding.

On admission to the hospital about 1½ months after birth, craniotabes and swelling of the costochondral junctions were evident. There was also suggestive enlargement of the epiphyses of radius and ulna. The roentgenograms of May 25, 1939 showed slight to moderate thickening of the long bones and cupping of the diaphyseal ends. The metaphyses were frayed and hazy, and some small decalcified areas were visible in the lower part of the femurs. Active, moderate rickets was diagnosed.

The calcium content of the serum per 100 cc. was 5.1 mg.; inorganic phosphorus, 5.06 mg.; phosphatase, 21.4 Bodansky units. The Wassermann reaction was negative.

The calcium content of the mother's serum per 100 cc. was 8.7 mg.; inorganic phosphorus, 3 mg.; phosphatase, 2.5 Bodansky units.

The child was treated with dihydrotachysterol (A.T. 10) in the following dosages: 3 drops daily from June 5 to 29, 1939; 8 drops daily, June 29 to September 26; the same dosage, October 4 to 27; 10 drops daily, December 18 to 22; 15 drops daily, January 8 to 13 ,1940. From May 13 to 28, 1940, the child received 15 drops daily of irradiated ergosterol (vigantol).

Although the child continued to suffer for many weeks from furunculosis, diarrhea, and fever, and remained in a critical condition for a long time, the rickets ultimately healed.

On July 15, a slight improvement of the rickets could be seen on the roentgenograms. Irregular calcification was still present in the ends of the diaphyses, but the haziness of the metaphyses was less marked. On August 19, condensation at the diaphyseal ends of the shafts became visible, indicating a slight improvement in the calcification of the bones. On Octo-

TABLE 1

SERUM ANALYSES OF AN INFANT WITH FETAL RICKETS TREATED WITH DIHYDROTACHYSTEROL

Date	Calcium mg./100 cc.	Inorganic phosphorus mg./100 cc.	Phosphatase units/100 cc.*
5/25/39	5.1	5.06	21.4
6/8/39	4.5	5.56	11.46
7/15/39	6.7	4.1	5.04
8/21/39	9.3	3.8	—
9/6/39	9.9	3.8	6.1
9/20/39	9.1	3.	14.7
10/10/39	7.2	4.14	10.4
11/27/39	8.	4.4	10.18
12/18/39	7.7	5.8	10.36
1/5/40	10.5	5.33	5.7
1/22/40	10.	5.3	5.4
3/12/40	8.8	4.2	5.2
5/27/40	10.5	7.3	5.7

* Bodansky units.

ber 28, the diaphyseal ends were more sharply defined and less irregular. The zone of condensation was somewhat increased, and the cupping of the diaphyseal ends was much reduced. The carpal and tarsal bones had also increased in size. The condition had improved, but the rickets was not yet arrested.

On January 22, 1940, the diaphyseal ends of the long bones had become sharper in outline, the flaring was less marked, and the zone of density was better defined. There was another slight increase in the size of the carpal and tarsal bones. On May 27, a small narrow zone of condensation with a slight ground glass appearance was found in the metaphyses. The deformity had almost completely disappeared, and considerable improvement of the rickets had been obtained.

The biochemical changes in the blood of this child are given in TABLE 1.

References

1. POMMER, G. 1885. Untersuchungen über Osteomalazie und Rachitis. Vogel. Leipzig, Germany.
2. SCHMORL, G. 1909. Ergeb. inn. Med. u. Kinderheilk. **4:** 403.
3. LOOSER, E. 1920. Deut. Z. Chir. **152:** 210.
4. PICK, L. 1932. Harvey Lectures. **27:** 179.
5. SNAPPER, I., I. FEDER & S. FALK. 1954. Ann. Int. Med. **93:** 807.
6. FANCONI, G. 1955. Metabolism. **4:** 95.
7. DENT, C. E. 1952. J. Bone and Joint Surg. **34B:** 266.
8. CHU, H. I. 1940. Chinese Med. **57:** 114.
9. FROMME, A. 1922. Ergeb. Chirurgie. **15:** 1.
10. SNAPPER, I. 1941. Chinese Lessons to Western Medicine. Interscience. New York, N. Y.

11a. MAXWELL, J. P. 1923. Chinese Med. J. **37:** 625.
11b. MAXWELL, J. P. 1930. Proc. Roy. Soc. Med. **23:** 639;
11c. MAXWELL, J. P. 1935. Proc. Roy. Soc. Med. **28:** 265.

12. MAXWELL, J. P., H. T. PI, H. A. LIN & C. C. KUO. 1939. Proc. Roy. Soc. Med. **32:** 287.
13. MILES, L. M. & C. T. FENG. 1925. J. Exptl. Med. **41:** 137.
14. CHU, H. I., T. F. YÜ, K. P. CHANG & W. T. LIU. 1939. Chinese Med. J. **55:** 93.
15. HANNON, R. R., S. H. LIU, H. I. CHU, S. H. WANG, K. C. CHEN & S. K. CHOU. 1934. Chinese Med. J. **48:** 623.
16. LIU, S. H., K. Y. CHIN, H. I. CHU & H. C. PAI. 1940. Chinese Med. J. **58:** 141.
17. LIU, S. H., R. R. HANNON, H. I. CHU, K. C. CHEN, S. K. CHOU & S. H. WANG. 1935. Chinese Med. J. **49:** 1.
18. LIU, S. H., H. I. CHU, C. C. SU, T. F. YÜ, & T. Y. CHANG. 1940. J. Clin. Invest. **19:** 327.

19a. CHU, H. I., S. H. LIU, T. F. YÜ, H. C. HSU, T. Y. CHENG & H. C. CHAO. 1941. J. Clin. Invest. **19:** 349.
19b. CHU, H. I., S. H. LIU, T. F. YÜ, H. C. HSU, T. Y. CHENG & H. C. CHAO. 1941. J. Clin. Invest. **20:** 255.

20. GREEN-ARMYTAGE, V. B. 1928. Indian Med. Gaz. **63:** 357.
21. VAUGHAN, K. 1939. Proc. Roy. Soc. Med. **32:** 297.
22. TSO, E. 1925. Chinese Med. J. **38:** 2.
23. CHU, F. T. & C. SUNG. 1940. J. Pediat. **16:** 607.
24. SNAPPER, I. 1955. Ann. N. Y. Acad. Sci. **63**(1): 92.
25. SNAPPER, I. 1949. Medical Aspects of Bone Diseases. 2nd ed. Interscience. New York, N. Y.

Part IV. Problems of Parturient Paresis in Dairy Cows

CALCIUM BALANCES AND CHANGES OF SOME BLOOD AND URINARY CONSTITUENTS AS RELATED TO PARTURIENT PARESIS IN DIARY COWS*

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Parturient paresis (milk fever) in dairy cattle is the result of a metabolic dysfunction at the time of parturition. The etiology of the disease is not well understood. The well-established physiological changes associated with this disease, as reviewed by Hibbs,[1] include hypocalcemia hypophosphatemia, hypermagnesemia, and a decrease in blood serum citric acid. Any differences in metabolism between cows that develop the disease and those that do not might shed light on the etiology of parturient paresis. A knowledge of the calcium balance before parturition should provide some clue to the nature of the disease and, to a lesser extent, knowledge of the balance for other minerals should be of value.

Parturition is a period of extreme stress in the dairy cow, and perhaps the adrenal glands are incapable of sufficiently rapid adjustment to the changes that occur at the time of parturition in the case of cows that develop parturient paresis. Changes in the blood and urinary levels of sodium and potassium are indicative of changes in adrenal function. The evidence is strong that the Jersey breed is more susceptible to this disease than are other dairy breeds. Primiparous animals are never attacked, and the disease is more prevalent among mature dairy animals.

The purpose of the experiment now described was to determine differences in the metabolism of some inorganic and organic constituents between cows that develop milk fever and those that do not.

Experimental Methods

The first phase of this study included 16 parturitions in 14 dairy cows. This herd consisted of 8 mature Jersey cows, 4 primiparous Jerseys, and 2 mature Holstein cows, and 2 mature Guernsey cows.

Twenty-four-hour collections of feces and urine were made on the estimated 30th, 15th, 10th, and last 5 consecutive days before parturition. Twenty-four-hour collections of feces, urine, and milk were made on the day of parturition, for 5 days thereafter, and again on the 10th, 15th, and 30th days postpartum. The feed intake was measured for 2 days before the collection day. Blood samples were drawn at the beginning and end of each collection day. Blood samples were drawn prior to treatment from all cows developing milk fever.

Feces were collected in a specially designed apparatus[2] and urine was collected at 2-hour intervals. All milk was removed from the udder either 2 or 3 times daily, and the calf was never allowed to nurse.

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Fecal and feed samples were wet-ashed with HNO_3 and HCl. Urine samples were filtered and diluted to the extent necessary for sodium and potassium analyses. All sodium and potassium analyses were made with a Beckman Model D U flame photometer. Calcium was determined on blood serum by the method of Clark and Collip.[3] The calcium content of urine, feces, feedstuffs, and milk was determined by the method of Morris *et al.*[4] Blood and urinary chlorides were determined by the method of Sendroy[5] and the chlorides of feces, feedstuffs, and milk by the method of Triebold.[6]

In the second phase of this study, blood samples were drawn from the jugular vein immediately before treatment for parturient paresis. All cows were diagnosed by a veterinarian as having the disease, and treated with a calcium borogluconate solution. Further blood samples were taken on the 5th, 10th, 15th and 30th days following treatment. By way of a control, blood samples were taken from normally calving cows shortly after parturition and at the same intervals following parturition as in the milk-fever group previously mentioned. Pyruvic acid was determined according to the method of Friedman and Hangen[7] lactic acid by the method of Barker and Summerson,[8] citric acid by the method of Taussky and Shorr,[9] and hematocrits according to the method of Wintrobe and Landsberg.[10] Analyses for all organic constituents were started within 2 to 3 hours after drawing the samples.

Results

Difficulty in accurately predicting the day of calving resulted in a paucity of prepartal information on some cows. To simplify presentation, the cows in this study were divided into 4 groups. Group I consisted of mature Jersey cows that calved normally; Group II, mature Jerseys that developed milk fever; Group III, primiparous Jerseys, and Group IV, mature Guernseys and Holsteins, in which group there were no cases of milk fever. Five parturitions were studied in Group I, 3 in Group II, 4 in Group III, and 4 in Group IV.

The average blood levels and total daily urinary excretion of K, Na, and chlorides for the 4 groups of cows are presented in FIGURE 1. Blood Ca levels are omitted, as they were found to be similar to those reported many times for normally calving and milk-fever cows. Urinary Ca excretion is not presented because levels were very low and bore no relation to parturition.

The average blood serum chloride levels of all groups tended to be higher near the day of parturition. A sharp drop to levels lower than those found prepartal occurred in all but the milk-fever group.

The blood Na level dropped near the time of parturition in all 4 groups. Group I showed an increase in the blood Na level on the day of parturition and a decrease on following days, Group II had the lowest levels on the day preceding calving, while Groups III and IV were lowest on the day of calving.

Blood K levels were not subject to the drastic fluctuations found for other constituents. Between 5 days prepartal and the day of parturition, blood K showed a general, if not a very marked increase. Group II showed a postpartum drop in K which was not found in other groups.

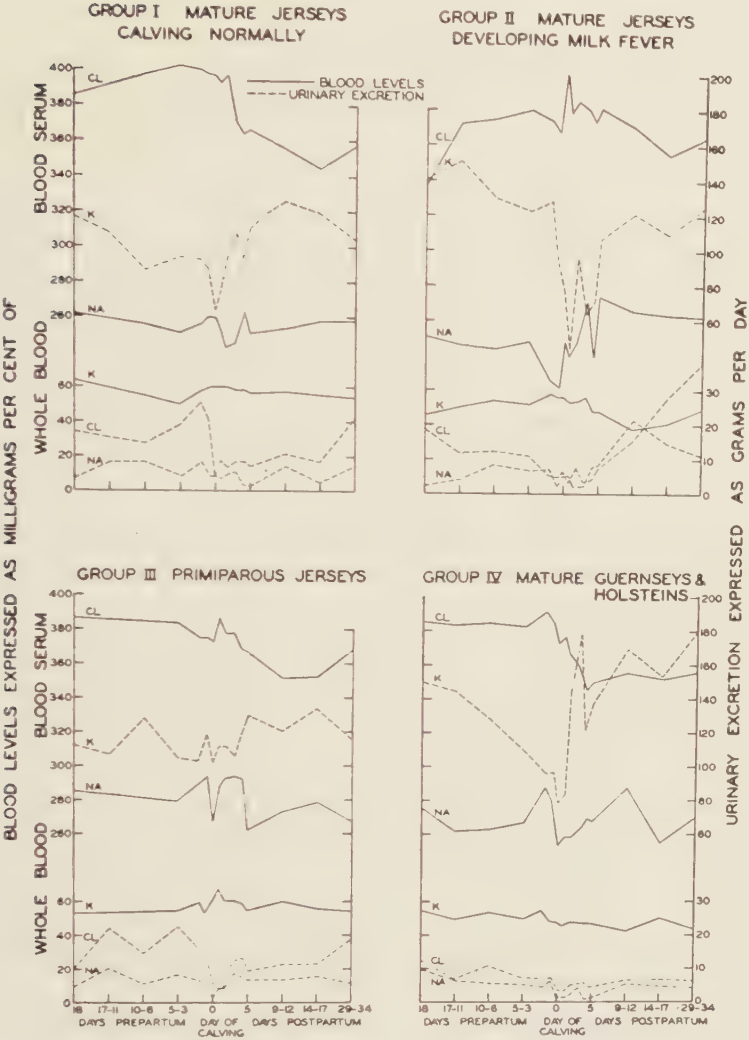

Figure 1. Blood and urinary levels of sodium, potassium, and chlorides for dairy cows at the time of parturition.

The urinary excretion of all minerals decreased at the time of parturition, despite a general increase in urine volume at this time. The volume of urine on the day of parturition ranged from 3.8 to 22.5 liters.

K accounts for most of the cation excreted in the urine. K excretion dropped sharply at the time of parturition in the milk-fever group. Group I showed a decline in excretion at this time. Group IV showed a decline that started 18 days prepartal and experienced a tremendous increase in excretion postpartum. Group II showed no marked changes in K excretion throughout the period.

Na and Chloride excretion in the urine was of a lesser magnitude than K excretion. The pattern of urinary Na excretion was similar for all groups with the exception of an increased level of excretion postpartum in cows developing milk fever. All groups exhibited a drop in Na excretion near the time of parturition.

Urinary chlorides decreased in all groups as parturition approached. This decline was abrupt in Groups I and III and more gradual in Groups II and IV. The postpartum urinary chloride picture was much the same except for the group developing milk fever, in which an increased excretion started at 9 to 12 days postpartum and continued until the end of the study.

FIGURE 2 presents the average mineral balances for the 4 groups of cows. Chloride balances are not presented because the information was incomplete.

The Ca balance was of major concern in this study and revealed some very interesting information. Prepartal Ca balances were consistently and strongly positive in the primiparous Jerseys (Group III), borderline for Groups I and IV, and definitely negative in the cows that developed milk fever (Group II). All cows were in a negative Ca balance following parturition. This was most pronounced in the milk-fever group.

The K balances closely paralleled the Ca balance in the prepartal period. In the postpartum period, Groups I and III had a definitely positive K balance, Group IV was slightly negative, and Group II was in a definitely negative balance.

Na intake and excretion was low compared to other constituents. The only obvious difference between groups was in the postpartum period when Groups II and IV were in a negative balance, while in groups I and III the intake and excretion were about equal.

The second phase of this study was concerned with the changes of various blood constituents associated with milk fever. TABLE 1 presents the data for 8 cows that developed milk fever, and TABLE 2 the data for 3 cows with normal parturitions. At the end of each table, the average blood levels for the groups are given for the five time intervals. Three cows suffered relapses and 2 cows had milk fever both prepartal and postpartum. The blood-serum Ca levels were as low or lower for these cows at the time of the second treatment as at the time of the first treatment.

Whole-blood Na levels were on the average higher, and K levels were slightly lower in pretreatment samples than in subsequent samples. Blood-serum chlorides also showed higher levels before treatment than at any later period. All of the cows, however, did not follow the pattern indicated by the average.

There also were marked differences in the organic constituents between pretreatment and posttreatment levels. Pyruvic acid levels were in all cases higher; lactic acid levels in most cases were also higher; citric acid levels were, with three exceptions, lower; and hemotocrits were higher in all but 1 case in the pretreatment samples, as compared to subsequent samples.

TABLE 2 presents the data for three cows that had normal parturitions. The blood-serum Ca levels were lower at the time of calving but not as low as the levels found in cows that were treated for milk fever. Whole-blood Na

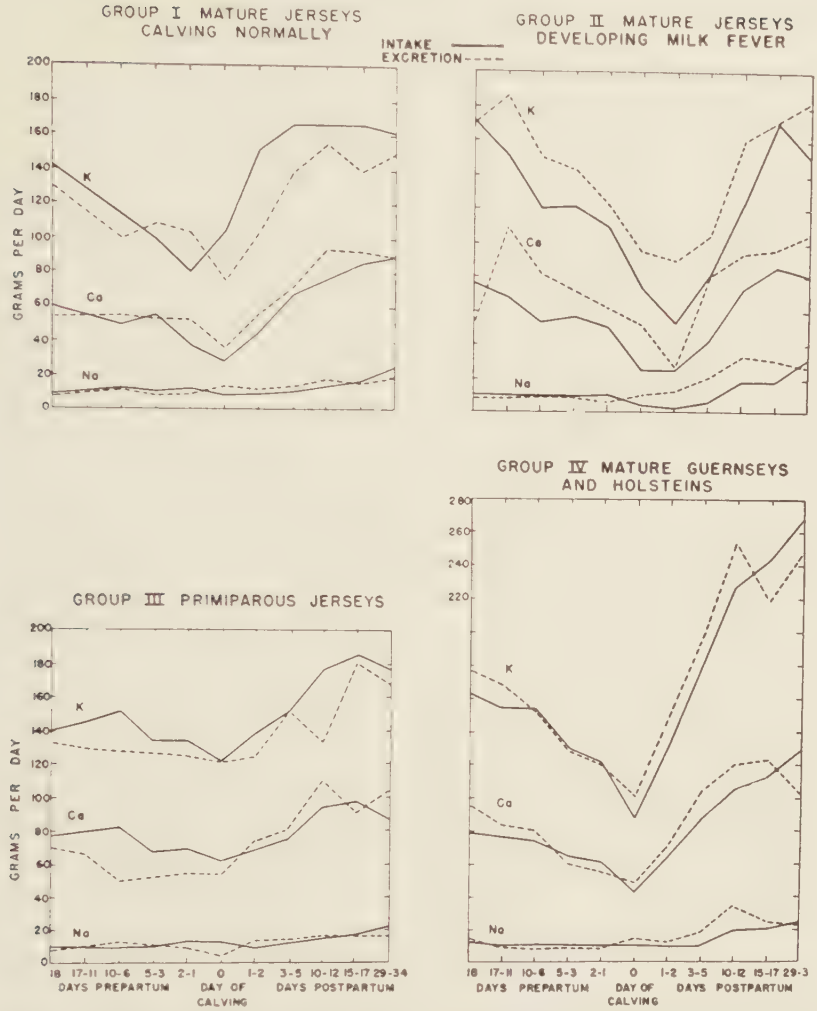

Figure 2. Intake and excretion of calcium, sodium, and potassium for dairy cows at the time of parturition.

levels were lower, and K and chloride concentrations were higher on the day of parturition than at subsequent times. The pyruvic-acid level was slightly higher, the levels of lactic and citric acids were slightly higher, and the levels of lactic and citric acids and hemotocrits were essentially the same, regardless of the time at which the samples were taken.

Discussion

The data presented indicate some interesting possibilities for speculation. The number of animals in each group is small, however, and, as mentioned previously, the prepartal information was meager in some cases. Further-

TABLE 1

BLOOD LEVELS OF CALCIUM, SODIUM, POTASSIUM, CHLORIDES, PYRUVIC ACID, LACTIC ACID, CITRIC ACID AND HEMATOCRIT IN COWS WITH PARTURIENT PARESIS

Cow No	Date	Blood serum calcium	Whole blood sodium	Whole blood potassium	Blood serum chloride	Whole blood pyruvic acid	Whole blood lactic acid	Blood serum citric acid	Hema-tocrit
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*%*
244*	1/4	6.1	256	61.3	333	2.30	—	5.3	44.8
	1/8	6.5	246	58.0	310	1.30	82.2	2.9	39.1
	1/14	8.6	249	52.6	323	1.53	27.7	4.3	—
	1/21	10.3	—	56.2	318	1.16	22.2	5.0	40.5
	2/4	10.4	270	51.8	404	1.30	13.2	4.2	39.8
266	1/9	4.4	240	53.3	—	2.86	140.4	2.0	45.0
	1/17	9.7	256	48.5	324	0.64	27.9	3.7	39.4
	1/21	8.8	232	48.2	318	0.83	18.4	5.1	40.0
	1/25	9.6	—	—	317	—	—	3.3	38.0
	2/12	9.7	277	45.3	361	1.06	12.1	6.8	34.8
243	2/12	4.2	246	47.4	346	1.90	82.8	2.8	48.5
	2/14	7.9	246	47.3	362	1.15	75.1	2.9	—
	2/16	8.4	262	44.0	337	0.97	25.9	3.9	43.2
	2/22	9.5	294	43.6	380	0.87	14.7	3.7	—
	3/6	10.6	261	44.0	362	0.78	35.9	5.7	39.8
	3/8	8.2	252	39.9	300	0.93	—	1.8	38.5
237	2/28†	4.8	251	55.6	374	—	—	5.6	49.7
	3/6	11.3	253	41.1	382	0.92	58.0	4.8	38.7
	3/14	10.0	302	45.4	358	1.16	52.5	4.7	37.7
	3/22	10.0	252	44.0	338	—	129.0	5.4	—
278	3/9†	5.6	246	48.5	389	1.88	17.6	7.0	47.5
	3/10‡	5.6	244	62.7	389	1.65	48.0	2.8	45.0
	3/14	10.0	255	44.4	358	1.50	170.0	4.8	40.0
	3/22	11.2	258	40.5	327	—	28.9	—	—
	4/8	—	261	57.7	357	1.06	32.8	—	41.2
259	4/1	6.8	253	40.8	379	2.80	175.4	1.5	42.0
	4/8	10.9	—	50.8	336	1.60	40.1	4.9	44.0
	4/13	11.4	254	62.7	355	1.01	18.8	5.9	39.7
	5/2	11.5	—	—	—	1.60	—	2.8	—
245	4/8	4.5	246	51.9	396	1.63	90.5	1.8	39.5
	4/9‡	4.1	164	48.5	368	1.44	46.0	1.7	—
	4/13	10.6	260	48.5	379	1.01	24.3	3.2	35.0
	4/18	10.5	266	42.7	—	—	12.2	—	—
	5/2	11.6	—	67.6	—	1.70	—	4.7	—
2116	1/28								
	5 pm	6.1	270	52.4	—	1.52	27.4	1.7	39.5
	10 pm‡	3.3	266	53.1	372	3.30	42.1	2.0	45.0
	2/1	10.5	262	40.1	395	1.38	—	—	36.2
	2/7	10.8	264	47.1	393	0.90	9.9	4.6	37.2
	2/12	10.7	263	43.9	367	0.99	15.9	4.9	36.0
	2/27	10.3	274	49.7	364	1.01	9.9	6.3	39.5
Av. pre-treatment level		5.0	244	52.3	372	2.13	74.5	3.1	44.6
4–6 d. post-treatment		9.7	256	46.0	360	1.24	73.6	4.1	38.8
8–14 d. post-treatment		10.0	265	47.8	347	1.02	24.1	4.5	38.8
15–22 d. post-treatment		10.4	259	47.0	340	0.98	25.7	4.7	36.1
24–32 d. post-treatment		10.2	267	52.0	357	1.24	39.4	4.6	38.8

* First date for each cow indicates pretreatment levels.
† Developed prepartal parturient paresis.
‡ Cow relapsed and was treated a second time.

TABLE 2

BLOOD LEVELS OF CALCIUM, SODIUM, POTASSIUM, CHLORIDES, PYRUVIC ACID, LACTIC ACID, CITRIC ACID AND HEMATOCRIT IN NORMALLY CALVING COWS

Cow No	Date	Blood serum calcium	Whole blood sodium	Whole blood potassium	Blood serum chlorides	Whole blood pyruvic acid	Whole blood lactic acid	Blood serum citric acid	Hematocrit
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
2117	12/29	7.9	246	69.1	434	1.38	14.4	3.7	44.1
	1/2	10.4	265	67.6	421	0.70	35.1	3.9	40.5
	1/7	10.7	241	62.9	323	0.88	35.9	3.4	41.3
	1/13	8.0	256	63.1	325	1.07	30.7	6.0	44.8
	1/28	9.4	260	50.8	359	0.80	19.1	3.6	—
2118	1/5	8.3	244	64.1	341	1.47	30.6	1.9	42.9
	1/10	9.8	230	59.0	295	1.08	27.0	3.6	46.8
	1/16	11.1	262	60.4	343	1.28	35.4	4.7	40.0
	1/20	8.3	279	59.8	334	1.01	27.2	5.8	46.8
	2/4	9.0	259	52.2	362	1.30	—	4.3	42.0
299	3/12	7.0	249	51.0	372	1.15	20.4	5.2	39.2
	3/16	10.9	266	44.3	346	1.40	14.7	3.2	44.0
	3/21	10.0	270	44.5	322	1.10	18.7	5.1	41.8
	3/26*	10.8	264	51.2	306	1.62	19.8	3.9	42.8
	4/8	10.4	266	41.9	366	1.05	17.5	7.0	36.5
Av. day of calving.		7.7	246	61.4	382	1.33	21.8	3.6	42.1
5 d. postpartum...		10.4	254	57.0	354	1.06	25.6	3.6	43.8
10 d. postpartum...		10.6	259	55.9	329	1.09	30.0	4.0	41.0
15 d. postpartum...		9.0	266	58.0	322	1.23	25.9	5.2	44.8
30 d. postpartum...		9.6	262	48.3	362	1.05	18.3	5.0	39.2

* Developed ketosis.

more, treatment of milk-fever cows with calcium gluconate may have altered the postpartum picture, particularly with respect to blood and urinary data.

The most interesting and probably the most important difference between groups is in the Ca balance prepartal. The cows that developed milk fever had a severe negative Ca balance for about 15 days prior to parturition, while the other groups maintained their Ca reserves and the primiparous Jerseys apparently were increasing their Ca stores. The strongly positive Ca balance for the primiparous group is most interesting when compared with the group that developed milk fever, for there is no authentic report of a case of parturient paresis in a primiparous animal. If this is a true picture of prepartal Ca metabolism of dairy cows, it probably indicates that those cows that develop milk fever either can not absorb Ca as efficiently from the intestine and/or excrete more Ca into the intestines than do normally calving cows. It has been shown by radiocalcium studies that high-level vitamin D therapy prepartal promotes Ca absorption but not retention.[11] The feeding of massive doses of vitamin D is an effective means of preventing milk fever.[12] A low-calcium, high-phosphorus ration will also prevent milk fever, and it is believed this is accomplished by stimulation of the parathyroid hormone production that mobilizes skeletal calcium.[13] The severe negative Ca balances found in the prepartal period of cows that develop milk fever indicate that these cows must be mobilizing Ca from the skeleton. This Ca is then lost through the intestines.

The observation that the K balance closely parallels that for Ca is difficult to explain. The severe negative K balance for the milk-fever group raises the question of the source of the K excreted above that supplied in the feed.

Na intake and excretion was about equal except for a rather decided negative balance in the milk-fever group in the postpartum period.

Blood levels and urinary excretion of Na and K, particularly the latter, are considered valuable indices of adrenal dysfunction in man and in laboratory animals. Increased Na and decreased K excretion in the urine generally indicates a decline in adrenal efficiency. It was thought that adrenal dysfunction might be associated with parturient paresis. Urinary data from this study, however, give no support to this postulate, because Na and K excretion both decreased near the time of parturition in the cows that developed milk fever. Twenty-four-hour collections were used for all urine analyses. Na and K analyses over a shorter period of time near the time of parturition and prior to treatment for milk fever perhaps would indicate a different trend.

Blood levels showing an increased K and a decreased Na are also an indication of adrenal insufficiency, but a less satisfactory one than urinary excretion data. FIGURE 1 shows a decreased Na level in all groups and a slight increase in blood K, except in 1 group, at or near parturition. TABLE 1 also shows that, on the average, the blood Na levels were somewhat lower and K levels higher just prior to treatment than at succeeding periods after treatment. TABLE 2 presents the data on 3 normally calving cows in which the same trend is apparent. If these changes can be interpreted as indicating adrenal involvement, it is apparently associated with the act of parturition rather than with parturient paresis.

Blosser and Smith[14] found that serum citric-acid levels decreased in all cows at the time of parturition, with a more precipitous drop in cows affected with milk fever. In this study, 3 of 8 cows did not have a decreased citric-acid level prior to treatment. The high levels of pyruvic acid prior to treatment represented the largest and most consistent changes associated with milk fever, except for Ca levels. The increased levels of pyruvic and lactic acid found in this study, together with the increase in blood glucose reported by Van Soest and Blosser,[15] indicate that a derangement in carbohydrate oxidation is associated with milk fever. Whether the changes in organic constituents as presented in TABLES 1 and 2 are basic to the etiology of milk fever or merely a result of inanition and a deranged mineral metabolism is not apparent from this study.

The data from TABLES 1 and 2 indicate that hematocrits are higher in milk-fever cows prior to treatment than in cows that calve normally. Van Soest and Blosser[15] found, however, that higher hematocrits were typical of all cows at the time of parturition.

The results discussed above are based on averages. It should be pointed out that in most instances all cows in a group did not follow the pattern indicated by the averages for that group.

Summary

This study concerns the average blood and urinary levels of Na, K, and chlorides on 16 parturitions in dairy cattle from 30 days prepartal to 30 days postpartum. Three of the parturitions were followed by attacks of parturient paresis. The total intake and excretion during this period of Na, K, and Ca is presented for the same animals. The blood levels of Ca, Na, K, chlorides, pyruvic, lactic and citric acids, and hematocrits are presented for 11 cows, 8 of which had milk fever and 3 of which calved normally. Blood samples were taken prior to treatment and at intervals afterwards.

The trend for average blood values near parturition was increased chlorides and K and lower Na. Differences between cows with milk fever and normal cows were not marked in this respect. Pyruvic and lactic acid levels were higher, citric acid was lower, and hematocrits higher in the milk-fever cows prior to treatment than at subsequent times.

The urinary excretion of all minerals decreased at the time of parturition. Urinary K and chloride excretion decreased more sharply in the milk-fever group than in other cows at parturition.

Cows that developed milk fever had severe negative Ca and K balances prepartal, while cows that calved normally were generally in a positive balance prepartal.

References

1. Hibbs, J. W. 1950. Milk fever (parturient paresis) in dairy cows. J. Dairy Sci. **33:** 758.
2. Ward, G. M., T. H. Blosser & M. F. Adams. 1953. Mineral balances at parturition as related to the occurrence of parturient paresis in dairy cows. Wash. Agr. Expt. Sta. Tech. Bull. 5.
3. Clark, E. B. & J. B. Collip. 1925. A study in the Tisdall method for the determination of blood-serum calcium with suggested modifications. J. Biol. Chem. **63:** 461.
4. Morris, H. P., J. W. Nelson & L. S. Palmer. 1931. Quantitave determination of calcium, magnesium and phosphorous in feedstuffs and cattle excreta. Ind. Eng. Chem. Anal. Ed. **3:** 164.
5. Sendroy, J. 1937. Micro determination of chloride in biological fluids with solid silver iodate, III. Titrimetric analysis. J. Biol. Chem. **120:** 335.
6. Triebold, H. O. 1946. Quantitative Analysis with Applications to Agricultural and Food Products. D. Van Nostrand Co., Inc. New York, N. Y.
7. Friedman, T. E. & G. E. Haugen. 1943. The determination of keto acids in blood and urine. J. Biol. Chem. **147:** 145.
8. Barker, S. B. & W. H. Summerson. 1943. Colorimetric determination of lactic acid in biological material. J. Biol. Chem. **138:** 535.
9. Taussky, H. H. & E. Shorr. 1947. A microcolorimetric method for the determination of citric acid. J. Biol. Chem. **169:** 103.
10. Wintrobe, M. M. & W. J. Landsberg. 1935. A standardized technique for the blood sedimentation test. Am. J. Med. Sci. **189:** 102.
11. Conrad, A. R., Sam T. Hansard, J. W. Hibbs & H. Patrick. 1955. Effects of massive oral doses of vitamin D upon the physiological behavior of radiocalcium and phosphorus in cattle. J. Animal Sci. **14**(4): 1198.
12. Hibbs, J. W. & W. D. Pounden. 1951. Recent developments in the use of vitamin D in the prevention of milk fever in dairy cattle. J. Dairy Sci. **34:** 498.
13. Boda, J. M. & H. H. Cole. 1954. The influence of dietary calcium and phosphorous on the incidence of milk fever in dairy cattle. J. Dairy Sci **37:** 360.
14. Blosser, T. H. & V. R. Smith. 1950. Parturient paresis. V. Blood serum levels of citric acid and calcium in normal parturient cows and cows with parturient paresis. J. Dairy Sci. **33:** 81.
15. Van Soest, R. J. & T. H. Blosser. 1954. A detailed study of levels of certain blood constituents in normally calving dairy cows and in dairy cows with parturient paresis. J. Dairy Sci. **37:** 185.

STUDIES ON PARTURIENT PARESIS IN CATTLE

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The majority of evidence accumulated through the years concerning the etiology of parturient paresis strongly indicates that this condition results from a failure of homeostatic factors, principally the parathyroid glands, that normally operate to maintain the blood calcium at a constant level in the face of the drain of calcium to the milk at the initiation of lactation.[5] On this basis, a rational approach to milk-fever prevention will involve either the preparation of these physiological mechanisms for optimal activity in advance of the demands for lactation or the temporary substitution of these mechanisms by artificial means until they can become sufficiently active to meet these demands by the adequate mobilization of calcium.

Calcium lost from the blood can be replaced in only two ways: either by increasing the intestinal absorption of calcium or by increasing the mobilization of previously deposited skeletal calcium. Attempts to prevent milk fever by increasing the calcium content of the diet have been ineffective, probably because intestinal absorption cannot meet the lactation requirement of the high-producing cow, particularly after a period of relatively low demand during the last portion of the preceding lactation and the dry period, and, in older animals, when the efficiency of calcium absorption may be reduced. Thus a better approach to milk-fever prevention seems to be the stimulation of bone-salt mobilization, either by activating the normal endogenous factors—the parathyroid glands—before lactation begins, or by the substitution of these factors by artificial means until the former can assert themselves.

The most practical method for causing parathyroid stimulation appears to be the use of low-calcium, high-phosphorus diets.[1] For the past several years we have studied the influence of such prepartal diets upon the incidence of parturient paresis in high-producing Jersey cattle. The results of these studies indicate that such diets will reduce significantly the occurrence of this condition in dairy cattle.

Experimental Studies

The animals used in this investigation were from several high-producing herds of Jersey cattle. Only cows that previously had calved at least twice were employed. During the last part of the dry period, usually the last 4 to 6 weeks, the cows were placed in dry lots and fed the various prepartal rations individually in stanchions. After parturition, the animals received the type of ration usually fed for milk production. It was necessary to use a low-calcium roughage (oat hay) and to restrict the amount fed during the prepartal period in order to obtain diets absolutely low in calcium. This required a relatively greater intake of concentrates so that the animals could obtain sufficient nutrients. Thus the low-calcium diets, and consequently the various control rations, had a low roughage-concentrate ratio necessitating the division of the daily ration: one half being fed in the morning, the other half in the evening.

Lactation records were obtained under the program of the Dairy Herd Improvement Association, Merced County, Calif., of most of the animals for the years immediately preceding and following the experimental period. Thus the influence of the various rations upon milk production could be determined. In most cases, blood samples were collected by venous puncture on the day of parturition and 4 days following, and were analyzed for total serum calcium[3] and phosphorus.[4] These determinations were made in order to diagnose milk fever definitely and to determine whether or not the low-calcium diets would have an influence on the postpartum levels of these blood constituents.

The influence of the prepartal low-calcium diets upon the incidence of milk fever was studied in 2 different trials. A description of the diets used and the daily calcium and phosphorus intakes are presented in TABLE 1. In Trial 1, 69 cows of one herd were divided randomly into 4 groups. Group 1 received alfalfa hay ad libitum during the entire dry period of 2 to 3 months in order to determine the incidence of milk fever in this particular herd when fed a prepartal high-calcium diet commonly employed under California conditions. Group 2 received an oat-hay, ground-barley ration supplemented by calcium carbonate in amounts sufficient to increase the dietary calcium-phosphorus ratio to a level equivalent to that of alfalfa hay. This control group was necessary in order to determine if the oat-hay and barley ration per se or the calcium and phosphorus intakes were the important factors in determining the incidence of milk fever. Group 3 received the same oat-hay and barley ration except that the dietary calcium-phosphorus ratio was adjusted to 1:1, a ratio usually considered to be balanced in this respect. Group 4 received a low-calcium diet supplemented by monosodium phosphate in order to decrease the calcium-phosphorus ratio. This group would consume only 5 pounds of

Table 1

Description of the Rations Employed and the Daily Calcium and Phosphorus Intakes for Trials on Milk-Fever Prevention

Trial no.	Group no.	Dietary calcium intake (gm. per day)	Dietary phosphorus intake (gm. per day)	Description of daily ration
1	1	—	—	alfalfa hay ad libitum
	2	120	34	4 lb. of oat hay, 10 lb. of ground barley containing 6.25% calcium carbonate
	3	34	34	4 lb. of oat hay, 10 lb. of ground barley containing 2% spent bone black
	4	6	20	5 lb. of oat hay, 5 lb. of ground barley containing 1.3% monosodium phosphate
	5	6	24	4 lb. of oat hay, 10 lb. of ground barley containing 1.3% monosodium phosphate
2	1	11	44	8 lb. of oat hay, 8 lb. of concentrate mixture* containing 1.5% monosodium phosphate
	2	11	74	8 lb. of oat hay, 8 lb. of concentrate mixture* containing 5% monosodium phosphate
	3	8.5–10	88–102	5–6 lb. of oat hay, 10–12 lb. of concentrate mixture* containing 5% monosodium phosphate

* Concentrate mixture composed of 40 per cent ground barley, 30 per cent rolled barley, 25 per cent wheat bran and 5 per cent cottonseed meal.

the ground barley, presumably as a result of the unpalatable phosphate supplement. It could be argued that any influence this ration might have on milk fever would be due to the reduction in food intake in comparison with the preceding control rations. In order to obviate this valid objection, a fifth group of cows was chosen from another high-producing Jersey herd. All of these animals had previous histories of milk fever and consumed the full daily ration of 4 pounds of oat hay and 10 pounds of barley supplemented with the phosphate salt.

Since the various diets used in Trial 1 were somewhat unpalatable and did not furnish sufficient nutrients for desired weight gains before parturition, a second trial was conducted in an attempt to find low-calcium rations that would correct these deficiencies and still prevent milk fever. Trial 2 involved 94 cows from 3 different Jersey herds, divided into 3 groups. The influence of the low-calcium prepartal diets upon the incidence of milk fever was assessed by comparing the number of cows with previous histories of this condition with its occurrence following the use of such prepartal rations. It is realized that milk fever at one parturition does not necessarily imply that this condition will occur again at a subsequent parturition but this is often the case.[6] The basic prepartal diets fed to all 3 groups of cows consisted of oat hay and a high-phosphorus concentrate mixture composed of ground barley, rolled barley, wheat bran, cottonseed meal, and supplemental monosodium phosphate. The amount of hay fed and the quantity of phosphate added to the concentrate mixture were altered in order to vary the dietary calcium and phosphorus intake. The rations fed to the cows of Trial 2 also are presented in TABLE 1.

Results and Discussion

The influence of the various prepartal rations used in the 2 trials on the incidence of milk fever and on the postpartum serum calcium levels are presented in TABLE 2. In Trial 1, the data indicate that a reduction in the prepartal dietary Ca:P ratio resulted in a decrease in the incidence of milk fever. In

TABLE 2
POSTPARTUM SERUM CALCIUM LEVELS AND THE INCIDENCE OF MILK FEVER FROM TRIALS ON MILK-FEVER PREVENTION*

Trial no.	Group no.	Number of cattle	Dietary calcium phosphorus ratio	Incidence of parturient paresis	Total serum calcium‡ (mg. per 100 ml.)	
					1st day postpartum	4th day postpartum
1	1	14	6:1	5	—	—
	2	19	5.9:1	5	8.89–1.03	9.87–0.68
	3	20	1:1	3	8.68–1.28	9.68–0.99
	4	16	1:3.3	0	9.53–1.12	10.18–1.01
	5	5 (5)†	1:4	0	—	—
2	1	26 (5)	1:4	2	8.73–1.03	10.34–0.77
	2	19 (10)	1:6.7	2	8.60–1.33	—
	3	49 (19)	1:10	3	8.65–1.24	10.23–1.18

* Compiled, in part, from data reported by Boda and Cole[1] and Boda.[2]
† Number of cattle with previous histories of parturient paresis.
‡ Excluding cows with outward symptoms of parturient paresis.

Group 1, 5 of 14; in Group 2, 5 of 19; in Group 3, 3 of 20; and in Groups 4 and 5, none, of the animals developed clinical symptoms of milk fever. The numbers of cases of this condition were the same in Groups 1 and 2, both of which received a Ca P ratio of approximately 6:1, demonstrating that the nature of the diet—that is, whether alfalfa hay or oat hay and barley—is not an important factor in determining the incidence of milk fever under these conditions. A reduction of the dietary Ca P ratio to 1:1 appeared to cause a fall in milk fever (Group 3), while a relatively low-calcium, high-phosphorus diet completely prevented this condition, even in cows with long previous histories of milk fever (Groups 4 and 5).

On the assumption that parathyroid stimulation would occur in the animals fed the low-calcium diets, it was thought that such diets might tend to prevent the postpartum fall of blood calcium that normally occurs at parturition. Although the mean levels on the day of parturition did appear higher in the animals fed a low-calcium diet (Group 4) as compared to those fed either high-calcium (Group 2) or normal-calcium (Group 3) diets, the differences between the means were not significant statistically.

In Trial 2 it is believed that the data indicate that the prepartal rations fed to Groups 2 and 3 definitely have lowered the incidence of milk fever. Of the 26 cows of Group 1, 5 of them having previous histories of milk fever, 2 exhibited symptoms of milk fever. Two of the 19 animals of Group 2, 10 having previous histories, and only 3 of the 49 cows of Group 3, 19 with previous histories, developed milk fever. In all cases, the symptoms of milk fever that did occur were not severe. Even though the number of cows of Group 1 with previous histories of this condition is small, it appears also that the prepartal ration fed to these animals has resulted in at least some reduction of milk fever. A number of the animals of this group were young and had calved only 2 or 3 times previously. Since milk fever is not common in such animals,[6] there was little opportunity for many of them to have had prior attacks. The various rations fed to the animals in this trial were palatable and allowed adequate weight gains before parturition. The rations had no adverse effects upon parturition, subsequent lactation, or the health of the calves.

In general, the results of Trial 2 agree with the previous trial in that a reduction of the incidence of milk fever accompanied a decrease in the prepartal dietary Ca P ratio. In comparing the occurrence of this condition in Groups 4 and 5 of Trial 1 with the number of cases in Group 1 of Trial 2, however, it seems that the absolute calcium intake may also be an important factor in milk-fever prevention. This is indicated by the fact that milk fever did not occur in the former groups that received a daily intake of only 6 gm. of calcium, while 2 cases occurred in the latter group receiving 11 gm., although the dietary Ca-P ratios were approximately the same in all groups.

From the practical standpoint, the data indicate that milk fever may be prevented by prepartal rations that are absolutely low in calcium in addition to containing a relatively large amount of phosphorus. If common livestock feeds are to be employed, it is necessary to restrict any roughage intake that decreases the palatability of the ration and thus limits the intake of nutrients. This may affect subsequent production. The data also indicate, however, that the incidence of milk fever may be greatly reduced, if not completely prevented, by prepartal rations that are only moderately low in calcium but contain a relatively large amount of phosphorus. This allows the formulation of palatable rations that will supply the nutrients required for pregnancy and lactation, and will effectively control parturient paresis.

Conclusion

Data are presented that indicate that low-calcium, high-phosphorus prepartal diets will reduce the incidence of milk fever in dairy cattle. It is believed that such diets exert this effect by activating the parathyroid glands, thus providing a means for the mobilization of skeletal calcium reserves. This provides sufficient calcium to meet the demands of lactation and to avert a severe fall of the blood-calcium level following parturition.

References

1. Boda, J. M. & H. H. Cole. 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever in dairy cattle. J. Dairy Sci. **37:** 360.
2. Boda, J. M. 1956. Further studies on the influence of dietary calcium and phosphorus on the incidence of milk fever. J. Dairy Sci. **39**(1)**:** 66–72.
3. Clark, E. P. & J. B. Collip. 1925. A study of the Tisdall method for the determination of blood-serum calcium with suggested modifications. J. Biol. Chem. **63:** 461.
4. Fiske, C. H. & Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. **66:** 375.
5. Hibbs. J. W. 1950. Milk fever (parturient paresis) in dairy cattle—a review. J. Dairy Sci. **33:** 758.
6. Metzger, H. J. & H. B. Morrison. 1936. The occurrence of milk fever in the Kentucky Station herd over a period of twenty years. Proc. Am. Soc. Anim. Production. **:** 48.

EFFECT OF PARTURIENT PARESIS AND THE ORAL ADMINISTRATION OF LARGE PREPARTAL DOSES OF VITAMIN D ON BLOOD CALCIUM AND PHOSPHORUS IN DAIRY CATTLE*

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Parturient paresis (milk fever) in dairy cattle is a metabolic disturbance typically associated with parturition and beginning lactation. It is characterized by low blood calcium and phosphorus, increased blood magnesium, paralysis, loss of consciousness and, if untreated, usually terminates in death. Although effective treatments, including udder inflation with air[1] and the intravenous injection of calcium salts[2] have been devised, the basic cause of milk fever has not yet been conclusively proved.

Among the numerous theories proposed regarding the etiology of milk fever,[3] the "parathyroid deficiency (hypocalcemia) theory" of Dryerre and Greig[4, 5] comes nearest to accounting for the immediate cause. These investigators reasoned that the lowered blood calcium in milk fever was the result of failure of the parathyroid glands to respond rapidly enough to replace the blood calcium lost through colostrum secretion. Although postulated by Dryerre and Greig,[4, 5] hypoparathyroidism has not yet been conclusively demonstrated in milk-fever cows. The evidence obtained by numerous investigators in support of the parathyroid deficiency (hypocalcemia) theory has been reviewed.[3]

In 1925 Little and Wright[6] first reported that blood-serum calcium was low in cows with milk fever, and that the severity of the symptoms was related to the degree of blood calcium diminution. Greig[7] later reported that in 82 cases of milk fever the minimum, maximum, and average blood-serum calcium level was respectively 3.00, 7.76, and 5.13 mg./100 ml.

The changes in blood-serum calcium, phosphorus, and magnesium that occur before and after parturition in normal and milk-fever cows are shown graphically in FIGURES 1 and 2.[8] Although the lowered serum calcium is the primary cause of milk fever, the increase in serum magnesium in relation to the low calcium may be responsible for the comatose symptoms usually observed, as opposed to the extreme hyperirritability seen in atypical cases of milk fever and in grass tetany where serum magnesium is below normal.

The low blood-phosphorus level in milk fever is of secondary importance and apparently results from (1) the simultaneous loss of both calcium and phosphorus from the blood into the mammary secretions in the form of a $(Ca) \times (PO_4) \times (HCO_3)_2$ complex, as suggested by Benjamin and Hess,[9] and (2) failure to be mobilized with the calcium from the skeletal reserves.

Data obtained in experiments conducted at the Ohio Agricultural Experiment Station[12] showed that milk-fever cows produced no more colostrum at

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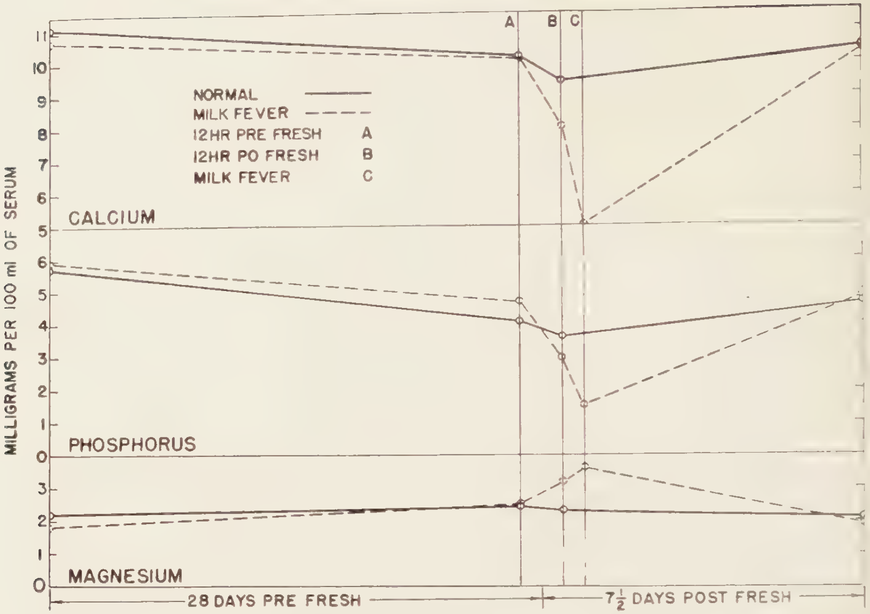

Figure 1. Changes in the serum calcium, phosphorus, and magnesium of the blood before and after parturition of 25 control Jersey cows fresh normally and 13 control Jersey cows that developed milk fever. Hibbs *et al.*

the first and second milking than cows that freshened normally. It was further shown[8, 12] that the colostrum of milk-fever cows contained no higher percentage ash, or calcium in the ash, than normally freshening cows. Thus no greater drain on blood calcium due to mammary secretion was experienced by milk-fever cows than by those that did not develop milk fever. This is further evidence that, primarily, milk fever is the result of temporary failure of the blood calcium regulatory mechanism to replace the blood calcium lost through colostrum secretion.

Thus in the treatment of milk fever the aim is to supply sufficient calcium to the blood to offset the loss through mammary secretion, and to maintain a normal blood level until the cow's own regulatory mechanism is able to respond. If this does not occur within a few hours, the injected calcium will be excreted and a relapse will occur. Table 1[12] shows the time between parturition and the occurrence of milk fever, and also the time between treatment of milk fever and relapses when they occurred.

Prevention of milk fever poses the problem of maintaining blood calcium at a normal or above normal level during the critical postpartum period. Two approaches to this problem under investigation at present are (1) prepartal parathyroid stimulation, being studied by Boda and Cole[13] at the California Agricultural Experiment Station, Davis, Calif., in which the parathyroids are presumably stimulated by feeding a low-calcium, high-phosphorus diet during the dry period, and (2) the "buffering" effect of increased intestinal absorption of calcium elicited by the prepartal feeding of large doses of vitamin D or other

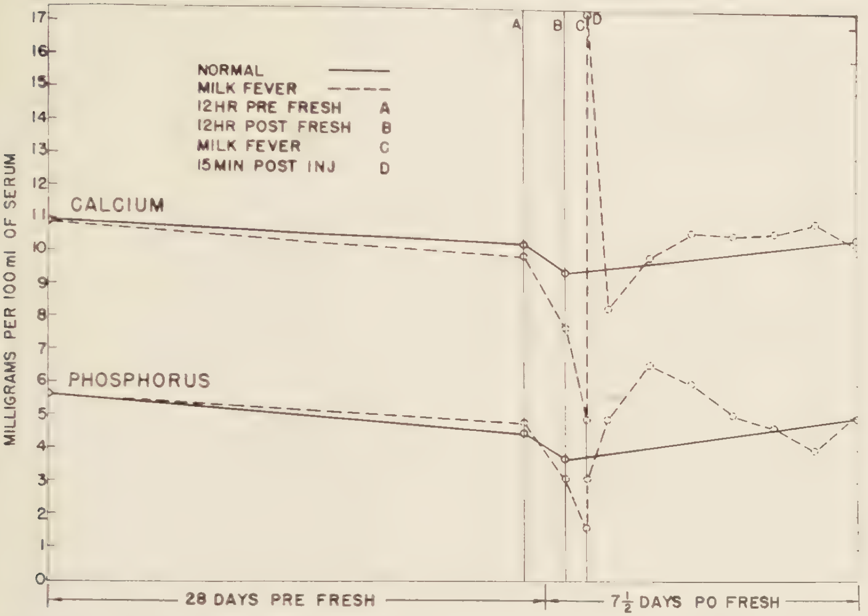

FIGURE 2. Changes in serum calcium and phosphorus in normal and milk-fever cows, showing detailed data following treatment of 1 milk-fever cow, using calcium gluconate intravenously. Hibbs *et al.*[8]

materials having calcemic (blood-calcium-raising) activity. Investigations conducted at the Ohio Agricultural Experiment Station have been based on the latter approach.[8, 11, 12, 13]

Both Greig[7] and Sjollema,[14] in 1930-1931, had suggested the possibility of using the calcemic action of vitamin D as a means of preventing milk fever, but neither presented experimental evidence of its value. The abundant availability of vitamin D in concentrated form and at relatively low cost gave promise of a practical material if an effective preventive procedure could be found.

In the initial studies[8, 11, 12] various amounts of vitamin D, 1, 2, and 5 million

TABLE 1*

THE LAPSE OF TIME BETWEEN PARTURITION AND MILK FEVER IN JERSEY COWS

Year	No. cases milk fever	Attack (hr. postpartum)	No. of relapses	Relapse (hr. post-treatment)
1941	2	14.0 ± 2.5	0	—
1942	11	20.0 ± 7.8	4	17.0 ± 5.3
1943	5	33.0 ± 13.3	1	29.0 ± 0.0
1944	8	20.0 ± 5.1	1	26.0 ± 0.0
1945	4	19.0 ± 7.8	0	—
1946	6	22.0 ± 4.6	2	24.0 ± 0.0
	36	21.7 ± 7.1	8	21.4 ± 2.7

* Hibbs *et al.*[12]

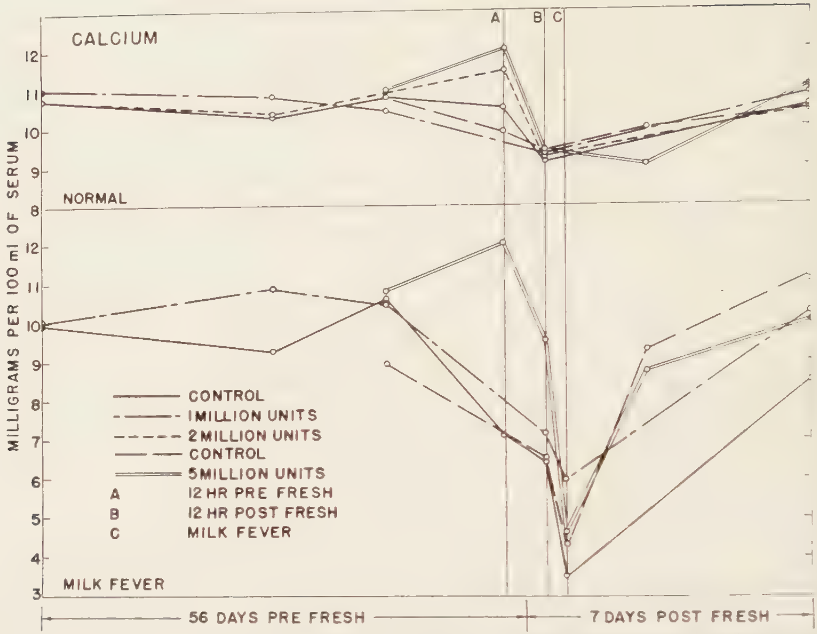

FIGURE 3. Changes in the prepartal and postpartum blood-serum calcium in normal and milk-fever cows fed, respectively, 1, 2, and 5 million units of vitamin D daily. In 1945 vitamin D was fed for one month prepartal. In 1946 vitamin D feeding was begun 2 weeks prepartal. Hibbs *et al.*[12]

units, were fed daily for 2 to 4 weeks prepartal and for 1 week postpartum, except in the case of the 5 million-unit level fed for only 3 days postpartum. The sources of vitamin D in these studies were 9-F irradiated dry yeast when fed at the 1 and 2 million-unit level, and viosterol (irradiated ergosterol in corn oil, 1 million units per gm.) when 5 million units per day were fed. The yeast was fed mixed with the grain, and the viosterol was administered in gelatin capsules, using a balling gun.

Both prepartal and postpartum changes in the blood calcium, phosphorus, and magnesium in cows fed, respectively, none, 1, 2, and 5 million units of vitamin D per day that freshened normally, and also those cows that had milk fever are shown in FIGURES 3, 4 and 5.[12] Although blood calcium was slightly increased before parturition in the normally freshening cows fed the higher doses of vitamin D, no differences in blood calcium existed between the controls and experimental cows within 12 hours after parturition, and no reduction in milk-fever incidence was observed.

The vitamin D content of the blood of cows fed, respectively, 1, 2, and 5 million units of vitamin D in these experiments[8, 12] was found to be, respectively, 2, 4, and 10 times that of the control cows. Thus, elevating the blood vitamin D level several times above normal did not provide sufficient calcemic effect to maintain the prepartal increase observed in the blood-calcium level during the critical 18 to 24 hour postpartum period.

Based on the results of Campbell and Turner,[15] it was reasoned[12, 13] that this

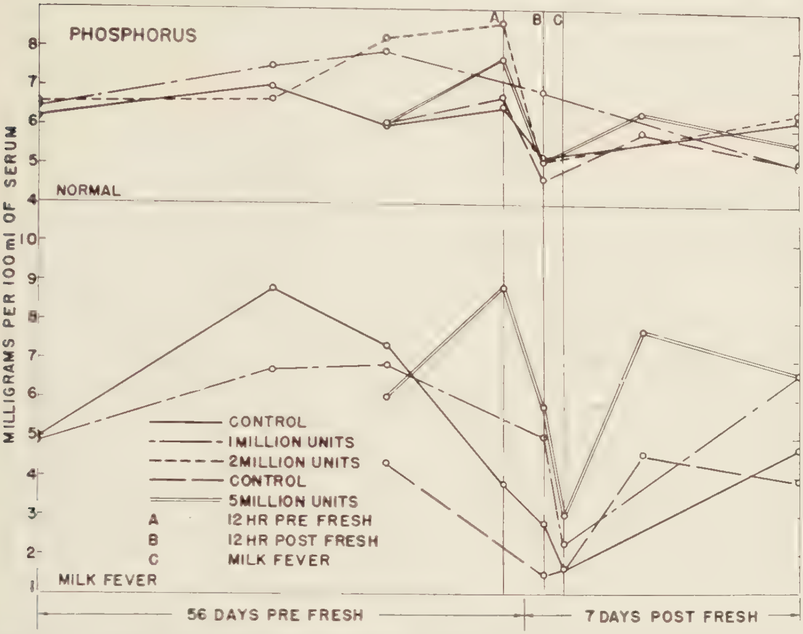

FIGURE 4. Changes in the prepartal and postpartum blood-serum phosphorus in normal and milk-fever cows fed, respectively, 1, 2, and 5 million units of vitamin D daily. In 1945 vitamin D was fed for 1 month prepartal. In 1946 vitamin D feeding was begun 2 weeks prepartal. Hibbs *et al.*[12]

lack of effect may have been due, at least in part, to suppression of parathyroid activity resulting from the prolonged prepartal elevation of blood calcium. Accordingly, a series of experiments was conducted[13] in which larger doses of vitamin D were fed for shorter periods prepartal.

Two sources of vitamin D were fed: (1) viosterol and (2) irradiated dry yeast (types 36-F and 142-F, containing 36,000 and 142,000 U.S.P. units per gram, respectively). The irradiated yeast was fed by premixing one half of the daily dose with the grain at each A.M. and P.M. feeding. The grain mixture containing the yeast was mixed with silage when silage was available. A few cows did not begin to eat the yeast soon enough and were excluded from the experiment. The viosterol was given in gelatin capsules with a balling gun. All cows were subjected to the usual feeding and management practices employed in the herd during the dry period. The roughage for the most part was medium to good quality legume hay, meadow crop, and corn silage, and pasture in season. Simple grain mixtures were fed in moderate amounts.

Some difficulty was encountered in predicting the calving date within the required range. Cows that freshened before 3 full days of vitamin D feeding or that failed to freshen within 1 day after termination of vitamin D feeding were excluded from the data. Cows that calved before 8 full days of vitamin D feeding were given 1 postpartum dose at the next feeding after parturition. Thus only the cows that received from 3 to 8 days of vitamin D feeding were included. Data were obtained from some cows at more than one parturition

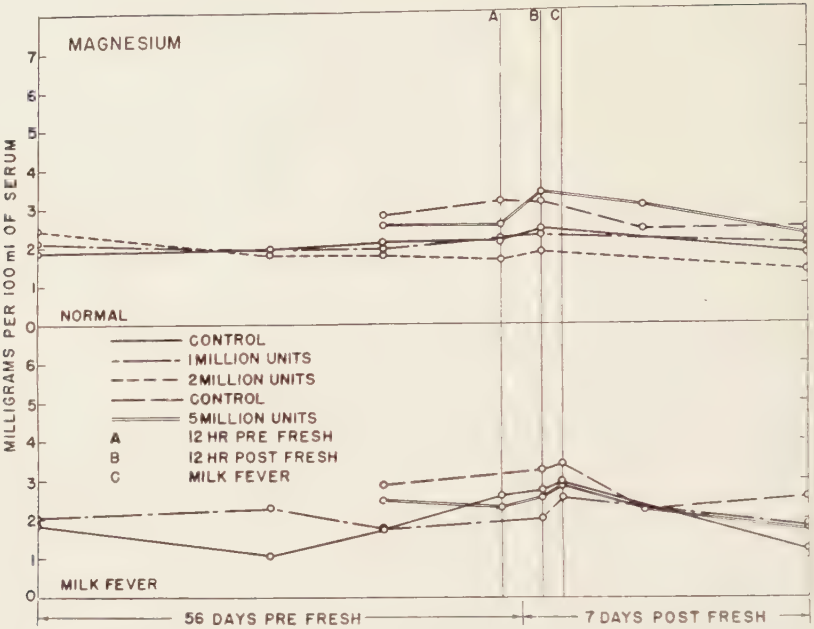

FIGURE 5. Changes in the prepartal and postpartum blood-serum magnesium in normal and milk-fever cows fed, respectively, 1, 2, and 5 million units of vitamin D daily. In 1945 vitamin D was fed for 1 month prepartal. In 1946 vitamin D feeding was begun 2 weeks prepartal. Hibbs *et al.*[12]

after different treatments. All cows were milked out as completely as possible at the next milking after parturition.

The milk-fever incidence data at parturitions after various levels of vitamin D feeding, in comparison to the controls, are presented in TABLE 2.[13] Both sources of vitamin D, irradiated dry yeast or viosterol, appeared to be equally effective. Because of the small numbers fed the 5- and 20-million-unit levels, it cannot be said with certainty what the minimum effective dosage would be. Some degree of protection against milk fever, however, appeared to result from all 4 levels of vitamin D feeding. Thirty million units of vitamin D per day fed for 3 to 8 days prepartal gave complete protection.

In TABLE 3[13] the average results of the blood analyses made at normal parturitions in which various amounts of vitamin D were fed for 3 to 8 days prepartal are compared with normal parturitions in which the cows were not fed vitamin D. Some of the control cows had been fed vitamin D prior to previous parturitions.

Complete chemical data were not available from all parturitions, and the number of parturitions represented in the averages are indicated in parentheses in TABLE 3. Complete serum calcium and phosphorous data are available from only 6 parturitions in which the cows were fed at the 10 million-unit level, from 3 at the 20 million-unit level, and from 4 at the 30 million-unit level. These data along with the control data from TABLE 3[13] are shown graphically in FIGURE 6.[13] The lowering of milk fever incidence at the 10 million-units-

TABLE 2*

EFFECT OF VARIOUS AMOUNTS OF VITAMIN D FED FOR A FEW DAYS PREPARTAL ON MILK FEVER INCIDENCE IN JERSEY COWS†

U.S.P. units of vitamin D fed daily 3-8 days prepartal	No. of parturitions	No. with milk-fever at previous parturitions	No. of milk fever cases	Per cent incidence of milk fever	Source of vitamin D fed
30 million	10	10	0	0	5 cows fed 142-F irradiated dry yeast‡ and 5 fed viosterol
20 million	4	4	0	0	viosterol§
10 million	12	7	1	8	viosterol§
5 million	5	2	1	20	36-F irradiated dry yeast‖
Controls—no vitamin D, no milk fever at previous parturitions	50	0	27	54	
Milk fever at previous parturitions	22	22	14	64	

* Hibbs and Pounden.[13]

† All cows had at least 2 previous lactations.

‡ 142,000 units per gm.

§ 1 million units per gm., irradiated ergosterol dissolved in corn oil.

‖ 36,000 units per gm.

per-day level of vitamin D feeding, and the complete protection resulting from the 20- and 30-million-unit level (TABLE 2),[13] were accompanied by the maintenance of blood calcium and phosphorus at levels higher than the controls during the critical postpartum period (TABLE 3 and FIGURE 6).[13]

The relatively high blood-calcium level maintained during the critical postpartum period is believed to be the cause of, or at least an indicator of, the protection against milk fever. The mode of action of vitamin D in eliciting the increased blood calcium, much of which was apparently due to ionic calcium,[17] however, is not completely understood.

In experiments conducted in cooperation with the Department of Veterinary Pathology, Ohio State University, Columbus, Ohio,[13, 17] the extremely low parathyroid activity, the increased gland size, and the histological changes in the parathyroid glands of cattle fed massive doses of vitamin D compared to the controls, indicated that the observed increase in blood calcium was not due to parathyroid stimulation.

Several experiments have been reported indicating that vitamin D increases intestinal absorption of calcium.[15, 19, 20] Thus the primary calcemic effect of massive doses of vitamin D appears to be the result of a parathyroid replacement action through which higher than normal blood calcium is maintained during the critical postpartum period, despite the hypoparathyroid state resulting from the vitamin D feeding.

These observations are not in support of the hypothesis of Taylor *et al.*[21, 22] working with dogs, in which the calcemic action of vitamin D was attributed to its stimulating effect directly on parathyroid tissue. Further, Taylor *et al.*[23] found that dogs made tolerant to repeated doses of parathyroid hormone were resistant to the toxic effects of large doses of vitamin D, even though a high

TABLE 3*

EFFECT OF VARIOUS AMOUNTS OF VITAMIN D FED FOR A FEW DAYS PREPARTAL ON THE BLOOD SERUM CA, P, AND MG OF NORMALLY FRESHENING JERSEY COWS†

U.S.P. units of vitamin D fed daily 3-8 days prepartal	Blood serum constituent	Approx. 1 wk. pre fresh (pre vit. D)	Within 12 hr. pre fresh	Within 12 hr. post fresh	Approx. 24 hr. post fresh.	Approx. 48 hr. post fresh.	Approx. 72 hr. post-fresh.	Approx. 1 wk. post fresh.
		mg. %	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
30 million	Ca	11.55 (9)‡	12.23 (8)	11.04 (10)	11.81 (9)	11.84 (8)	12.29 (5)	
	P	5.94 (5)	8.09 (6)	6.96 (6)	7.24 (6)	8.87 (6)	10.49 (4)	
	Mg	1.93 (5)	2.09 (6)	2.36 (7)	2.27 (7)	2.39 (7)	1.70 (5)	
20 million	Ca	11.79 (3)	11.83 (3)	11.50 (3)	12.70 (3)			
	P	4.83 (3)	5.90 (3)	7.68 (3)	6.91 (3)			
	Mg		2.72 (3)	2.25 (3)	2.09 (3)			
10 million	Ca	10.34 (11)	10.88 (7)	9.76 (11)	9.95 (10)			9.48 (4)
	P	4.58 (10)	6.44 (7)	4.57 (10)	4.82 (11)			5.62 (4)
	Mg	2.31 (7)	2.31 (3)	2.38 (7)	2.25 (7)			
5 million	Ca	11.44 (2)	11.84 (2)	11.89 (2)	11.95 (1)			11.32 (2)
	P	6.14 (2)	5.18 (2)	5.41 (2)	4.52 (1)			3.89 (2)
	Mg	2.40 (2)	2.62 (2)	1.92 (2)	1.12 (1)			2.14 (1)
Controls (No vitamin D)	Ca	10.32 (7)	9.90 (5)	8.72 (8)	8.62 (5)			10.31 (4)
	P	5.49 (7)	4.28 (3)	4.44 (7)	4.91 (7)			5.88 (4)
	Mg	2.14 (6)	2.19 (4)	2.65 (7)	2.69 (4)			1.70 (4)

* Hibbs and Pounden.[13]

† All cows had at least 2 previous lactations.

‡ Number of parturitions in the average indicated in parentheses.

level of blood calcium persisted for several weeks. This apparent lack of agreement may be associated with the recognized resistance of the bovine to the calcemic action of vitamin D as compared to that of the dog and of some other animals.

The marked increase in serum phosphorus at these high levels of vitamin D feeding (TABLE 3 and FIGURE 6),[13] although apparently not directly concerned with milk-fever prevention, may be indicative of the parathyroid depressing effects of vitamin D. Parathyroid gland removal has been shown by Talmadge *et al.*[24] to cause a rise in serum phosphorus that was restored to normal by parathormone injection.

As the calcemic effect of vitamin D decreases following the termination of vitamin D feeding the hypoparathyroid glands apparently respond rapidly enough to the stimulus of milk secretion to prevent hypocalcemia from developing.

Toxicity studies were carried out in cooperation with the Departments of Veterinary Pathology and Veterinary Physiology and Pharmacology, College

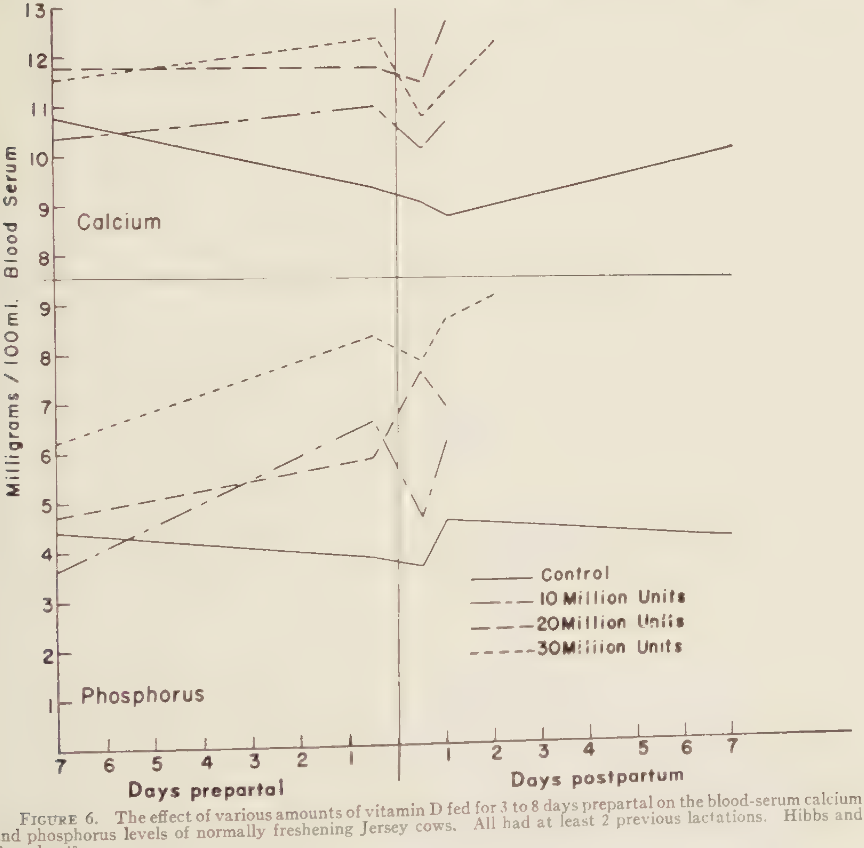

FIGURE 6. The effect of various amounts of vitamin D fed for 3 to 8 days prepartal on the blood-serum calcium and phosphorus levels of normally freshening Jersey cows. All had at least 2 previous lactations. Hibbs and Pounden.[13]

Table 4*

Effect of Feeding 30 Million Units of Vitamin D Daily for 7 Days Prepartal on the Vitamin D Content of Colostrum and Milk

No. of cows	Day sampled postpartum	Av. vitamin D/gm. butterfat	Av. vitamin D/qt. milk†
		i.u.	*i.u.*
1‡	1st	300	12,000
4	3rd–4th	93	3,720
4	6th–7th	55	2,200

* Hibbs and Pounden.[13]
† Estimated assuming 40 gm. of butterfat per quart of milk.
‡ Two other first day samples assayed less than 300 units per gram; but exact amount was not determined

of Veterinary Medicine, Ohio State University, Columbus, Ohio, to determine the safe limitations of using these large doses of vitamin D for milk-fever prevention, taking into consideration both daily dosage and duration of feeding.[13, 17] When 30 million units of vitamin D were fed, no serious pathological effects were observed when administration was limited to 10 days or less. Prolonged feeding of 30 million units of vitamin D per day for from 21 to 30 days, however, resulted in definite signs of toxicity. On this basis it seemed wise to limit the feeding of vitamin D to 7 days or less in order to avoid possible harmful effects and to provide a margin of safety.

Experience indicates that little protection can be expected until vitamin D has been fed for at least 3 days and for not longer than 1 or 2 days after vitamin D feeding is stopped. The prediction of the data of parturition within these relatively narrow limits poses a problem that cannot be completely overcome without more accurate means for determining the calving date in advance. The known factors that influence length of gestation in cattle have recently been reviewed by Brakel *et al.*[16]

As indicated in table 4,[13] the vitamin D content of the colostrum and milk declined rapidly during the first few days. Vitamin D-fortified milk normally contains at least 400 units per quart, and much higher therapeutic doses are often given than would reasonably be expected to be consumed from drinking the milk of vitamin D-treated cows for a short time. These levels of vitamin D are therefore not considered to be a health hazard either to humans or to calves. On a practical basis, this preventive treatment would be used most advantageously on cows with previous histories of milk fever or on cows with at least two previous lactations in herds where a high incidence of milk fever had been experienced.

References

1. Schmidt, J. J. 1897. Studies og forsog over kalvningsfeberens, aarsag og behandling Maanedsskrift fur Dyrlaeger **9:** 228. Translated, Vet. Record 1902–03. **15:** 210.
2. Greig, J. R. 1930. Calcium gluconate as a specific in milk fever. Vet. Record **10:** 115.
3. Hibbs, J. W. 1950. Milk fever (parturient paresis) in dairy cows—a review. J. Dairy Sci. **33:** 758.
4. Dryerre, H. & J. R. Greig. 1925. Milk fever: Its possible association with derangements in the internal secretions. Vet. Record **5:** 225.
5. Greig, J. R. 1930. The nature of milk fever. Eleventh Int. Vet. Cong. **2:** 1.
6. Little, W. L. & N. C. Wright. 1925. The aetiology of milk fever in cattle. Brit. J. Exptl. Pathol. **6:** 129.

7. Greig, J. R. 1930. Studies in the prevention of milk fever. Vet. Record. **10:** 301.
8. Hibbs, J. W., W. E. Krauss, W. D. Pounden, C. F. Monroe & T. S. Sutton. 1946. Studies on milk fever in dairy cows. II. The effect of vitamin D on some of the blood changes in normal and milk-fever cows at parturition. J. Dairy Sci. **29:** 767.
9. Benjamin, H. R. & A. F. Hess. 1933. The forms of calcium and inorganic phosphorus in humans and animal sera I Normal, rachitic, hypercalcemia and other conditions. J. Biol. Chem. **100:** 27.
10. Boda, J. M. & H. H. Cole. 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever in dairy cattle. J. Dairy Sci. **37:** 360.
11. Hibbs, J. W., W. E. Krauss, C. F. Monroe & T. S. Sutton. 1946. Studies on milk fever in dairy cows. I. The possible role of vitamin D in milk fever. J. Dairy Sci. **29:** 617.
12. Hibbs, J. W., W. D. Pounden & W. E. Krauss. 1951. Studies on milk fever in dairy cows. II. Further studies on the effect of vitamin D on some of the blood changes at parturition and the composition of colostrum in normal and milk-fever cows. J. Dairy Sci. **34:** 855.
13. Hibbs, J. W. & W. D. Pounden. 1955. Studies on milk fever in dairy cows. IV. Prevention by short-time, prepartum feeding of massive doses of vitamin D. J. Dairy Sci. **38:** 65.
14. Sjollema, B. 1932. Nutritional and metabolic disorders in cattle. Nutrition Abstr. & Revs. **1:** 621.
15. Campbell, I. L. & C. W. Turner. 1943. The relationship of the endocrine system to the regulation of calcium metabolism. Mo. Agr. Expt. Sta. Bull. 352.
16. Brakel, W. J., D. C. Rife & S. M. Salisbury. 1952. Factors associated with the duration of gestation in dairy cattle. J. Dairy Sci. **35:** 179.
17. Wagner, A. R., C. R. Cole & J. W. Hibbs. Unpublished data.
18. Nicolaysen, R. 1951. The influence of vitamin D on the absorption of calcium from the intestine. Experiments with isolated loops. Acta. physiol. scand. **22:** 260 (Nutrition Abstr. & Revs. **21:** 306. 1951).
19. 1955. Vitamin D and Ca absorption. Nutrition Revs. **13:** 271 (Review of the work of B. B. Migicovsky and J. W. S. Jamieson. 1955. Can. J. Biochem. and Physiol. **33:** 202).
20. Conrad, H. R., S. L. Hansard, J. W. Hibbs & H. Patrick. 1955. Effects of massive oral doses of vitamin D upon the physiological behavior of radiocalcium and phosphorus in cattle. J. Animal. Sci. **14:** 1198.
21. Taylor, N. B., C. B. Weld, H. D. Branion & H. D. Kay. 1931. A study of the action of irradiated ergosterol and of its relationship to parathyroid function. Can. Med. Assoc. J. **24:** 763.
22. Taylor, N. B., C. B. Weld & J. F. Sykes. 1934. The relation of the parathyroid glands to the action of irradiated ergosterol. Proc. Roy. Soc. London. **116:** 10.
23. Taylor, N. B., C. B. Weld & J. F. Sykes. 1936. Parathormone tolerance in dogs. Brit. J. Exptl. Pathol. **17:** 104.
24. Talmadge, R. V., F. W. Kraintz, R. C. Frost & L. Kraintz. 1953. Evidence for a dual action of parathyroid extract in maintaining serum calcium and phosphate levels. Endocrinology. **52:** 318.

URINARY CALCIUM EXCRETION AND BLOOD CALCIUM LEVELS IN THE BOVINE NEAR THE TIME OF PARTURITION*

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Parturition in the dairy cow is a time of tremendous physiological activity. During this period hormonal levels are constantly changing, for the cow not only loses the placenta, a site of hormone production, but also is preparing to secrete large quantities of milk. The latter procedure causes the cow to secrete many food nutrients at a rate much faster than she can consume them in her diet. It is understandable that complications associated with parturition and the onset of lactation should occasionally occur.

It would be fortunate indeed if one could assume that hormonal balances and mineral metabolism at parturition and during lactation in the dairy cow were the same as that in other species. The dairy cow, however, a ruminant bred for centuries for high milk production, is different in several respects from humans and small experimental animals. Accordingly those engaged in studies of physiology of the dairy cow must, of necessity, study the dairy cow herself rather than draw inferences from studies of other species.

Calcium metabolism in the bovine has always been of special interest to those engaged in research with dairy cattle because of the sizable outputs of calcium in the milk for relatively long periods of time. Added impetus was given to studies in this area when it was demonstrated about 30 years ago[12] that a marked hypocalcemia existed during parturient paresis (milk fever). In spite of this, basic information on calcium metabolism, even in the normal cow, has been slow in accumulating.

The specific cause of the hypocalcemia in milk fever is not known. Many have related it to the sudden great demand for calcium associated with the initiation of lactation. While these sizable losses of calcium are undoubtedly associated with a hypocalcemia, the onset of lactation cannot be considered the sole cause of milk fever, for many heavy producing cows do not develop this syndrome at parturition.[19]

Practical dairymen are vitally interested in an effective means of preventing this disorder. Recent research along these lines in Ohio[20] and California[9] is very encouraging. Some progress is also being made toward an understanding of calcium metabolism in the normally lactating and in the parturient bovine. This type of information is necessary before the etiology of parturient paresis can be understood.

In this paper some of the more recent studies on calcium levels under varying conditions in the blood and urine of normal parturient cows and cows with parturient paresis will be discussed. In some cases we have taken the liberty of condensing data originally published in order to facilitate presentation.

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* Published with the approval of the Director, Washington Agricultural Experiment Stations, State College of Washington, Pullman, Wash.

TABLE 1

CHANGES IN THE TOTAL BLOOD SERUM CALCIUM AT PARTURITION IN NORMALLY-CALVING PREPARTAL-MILKED COWS[32]

Total blood serum calcium (mg.%)

Days prepartal			Day of parturition	Days postpartum		
3	2	1		1	2	3
10.1	10.0	9.9	8.6	8.6	9.7	10.1

Average of 37 cows.

Effect of Prepartal Milking on Blood Serum Calcium Levels

The first of a series of studies on parturient paresis made at the University of Wisconsin, Madison, Wis.,[6, 7, 25, 26, 27, 32, 33] dealt with the influence of prepartal milking on this disorder. Prior to the time this study was made it was suggested[26] that milk fever might be prevented by milking the cow several days before the expected date of parturition. The logic behind this practice was that a gradual initiation of lactation, started several days before parturition, would cause a less drastic change in calcium requirements at the critical period near parturition than would conventional management.

To test this theory, Smith and Blosser[32] prepartal-milked 46 Jersey cows from a large herd in North Carolina. Results are shown in TABLE 1.

The incidence of parturient paresis in this herd during the period of prepartal milking was no different statistically than during corresponding periods prior and subsequent to the conduct of the experiment. Blood serum calcium levels at the time of parturition in the normally-calving cows were typical of those normally found in nonprepartal milked cows.

Effect of Incomplete Milking on Blood Serum Calcium Levels

A method commonly suggested for controlling milk fever is the incomplete milking of cows following parturition. The logic behind this suggested method of prevention is obvious: namely, with incomplete milking, demands for calcium for milk production are smaller since presence of milk in the udder inhibits maximum milk secretion.

To test this theory, Smith and Niedermeier[33] subjected 56 multiparous cows to an experiment in which every other cow in the University dairy herd, following calving, was completely milked (with the aid of an injection of 10 I.U. oxytocin) while, in the remaining half of the herd, only about two thirds of the milk was withdrawn. Results of this experiment are shown in TABLE 2.

This experiment demonstrated that incomplete milking at parturition had no noticeable influence on blood serum calcium levels nor upon the incidence of parturient paresis.

An interesting corollary to this study was another reported by Niedermeier and Smith[25] that involved investigation of cows not milked following parturition. Four multiparous Jersey cows were used in this experiment. All cows showed an appreciable drop in blood serum calcium on the day of parturition,

TABLE 2

BLOOD SERUM CALCIUM LEVELS AT PARTURITION IN PARTIALLY VS. COMPLETELY MILKED COWS.[33]

Total blood serum calcium (mg.%)

	Days prepartal					Day of parturition	Days postpartum				
	5	4	3	2	1		1	2	3	4	5
Av. of 27 completely milked cows	10.2	10.3	10.2	10.3	9.9	8.3	8.4	9.1	9.6	9.8	10.0
Av. of 29 partially milked cows	10.2	10.2	10.4	10.2	9.9	8.2	8.5	9.4	10.1	10.1	10.0

Three cases of milk fever occurred in the completely milked group, 8 cases in the partially milked group.

even though they were not milked. One cow developed parturient paresis 36 hours postpartum. Blood serum calcium levels returned to normal by the second day postpartum.

Effect of Mastectomy on Blood Serum Calcium Levels

Parturient paresis in dairy cows is normally attributed to stresses associated with the initiation of lactation. If mastectomized cows developed milk fever, this logic could be considered invalid. To study the effect of mastectomy on blood serum calcium levels following parturition, Niedermeier *et al.*[27] mastectomized 4 Jerseys and 1 Guernsey cow, most of these animals having previous histories of milk fever. Following mastectomy, these cows were bred and were paired with cows comparable as to age, number of parturitions, and date of parturition. Studies were made of the mastectomized and normal cows at parturition. Results, based on averages, are shown in TABLE 3.

None of the cows developed parturient paresis. The control cows exhibited the characteristic decrease in blood serum calcium at parturition reported by many workers. The mastectomized cows exhibited little change as compared with the cows calving with intact udders. Neither blood serum magnesium nor blood plasma phosphorus values exhibited as much deviation from normal in the mastectomized as in the normal cows.

Effect of Udder Inflation on Blood Serum Calcium and Plasma Phosphorus

Prior to the time that calcium borogluconate was first suggested as a treatment for parturient paresis, this disorder was treated effectively by inflation of the udder with air. In some areas this method is still used, particularly in cows that do not respond well to the injection of calcium solutions. The *modus operandi* of udder inflation in curing milk fever is not definitely known. Some[13, 23] attribute its effectiveness to cessation of milk secretion. Others[10, 16] postulate that udder inflation gives rise to afferent stimuli responsible for the curative effects.

To study effects of inflation upon recovery following milk-fever attacks and on blood levels of certain inorganic constituents, Niedermeier and Smith[26] used 7 cows with milk fever that had been treated by udder inflation (pressures of 60 to 70 mm. Hg as measured by an aneroid manometer). Blood samples were taken pre-inflation and at frequent intervals following inflation for 20 hours. Results of this study, based on averages of 7 cows, are presented in TABLE 4.

TABLE 3

BLOOD SERUM CALCIUM BEFORE AND AFTER PARTURITION IN MASTECTOMIZED AND INTACT COWS (AV. OF 5 COWS IN EACH GROUP)[27]

Blood serum calcium (mg.%)

	Days prepartal			Day of parturition (Hours postpartum)			Days postpartum		
	3	2	1	1-3 hrs.	5-7 hrs.	9-11 hrs.	1	2	3
Mastectomized	9.9	10.4	10.3	10.2	9.9	10.3	10.7	10.9	11.0
Control	10.9	10.8	10.4	8.8	8.7	8.6	8.5	8.9	9.7

TABLE 4

EFFECT OF UDDER INFLATION ON BLOOD CALCIUM AND PHOSPHORUS IN COWS WITH PARTURIENT PARESIS[26]

	Preinflation	Hours postinflation								
		0.5	1.5	3.0	5.0	8.0	11.0	14.0	17.0	20.0
Ca	4.2	4.6	5.0	5.1	5.5	6.0	6.2	6.4	6.5	6.6
P	1.0	1.4	1.7	1.7	2.0	2.3	2.6	3.0	3.0	3.3

Blood constituents expressed as mg. per cent blood serum calcium and blood plasma phosphorus; average of 7 cows.

Afflicted cows were able to rise in 3 to 14 hours following treatment. Recovery was slower than is usually experienced following injection of calcium solutions. Recovery was noted in most cows when blood serum calcium values rose to about 6.0 mg. per cent. Both blood serum calcium and plasma phosphorus values rose slowly during the first 20 hours postinflation but, percentagewise, phosphorus recovered more rapidly than calcium. In none of the cows were blood serum calcium values completely normal within 20 hours postinflation. Blood serum magnesium levels were higher than normal in nearly all cows at the time of inflation, and they tended to decline slowly following treatment.

Blood Serum Calcium and Citric Acid Levels at Parturition

The relation between citric and calcium metabolism has drawn the attention of some investigators in recent years. Dickens[11] found that the hard substance in bone contained a large store of citric acid. Nicolaysen and Nordbö[24] reported that a lack of vitamin D resulted in a decrease in bone citric acid content. On the other hand, Steenbock and Bellin[33] demonstrated an increase in citrate content of blood, bone, kidney, heart, and the small intestine in rats given physiological doses of vitamin D. Similar results were obtained in dogs by Freeman and Chang.[15]

Alwall[3] obtained a parallel rise in blood serum calcium and citric acid in dogs with the injection of parathormone. Hebert[18] found elevated blood citric acid levels in human parathyroid osteitis, but observed that similar values were sometimes found in other diseases of bone. Perkins and Dixon[25] demonstrated a lowered bone citrogenase activity several weeks after parathyroidectomy but found no change in bone citrate due to parathyroid ablation.

An interesting relation exists between some of the steroidal hormones and calcium and citric acid metabolism. Shorr *et al.*[30, 31] have shown an increased urinary citric acid excretion associated with higher-than-average levels of estrogen in women. Klotz *et al.*[21] found that administration of natural estrogen reduced blood serum calcium in parathyroidectomized dogs but not in non-operated dogs. Duckworth and Ellinger[14] could find no effect on calcium retention following ovariectomy in rats. Pincus *et al.*[29] were able to increase blood serum citric acid in rabbits approximately 30 per cent by injection of ACTH, while blood serum calcium was lowered during the same interval. On the other hand, injection of cortisone caused a parallel decrease in both calcium and citric acid.

In an attempt to determine whether blood serum citric acid values paralleled blood serum calcium values at parturition in dairy cows, several studies were conducted.[6, 8, 12] Blosser and Smith[6] demonstrated a highly significant correlation between blood serum citric acid and calcium levels from 10 days prepartal to 10 days postpartum. The results of this study, based on 33 normally-calving cows and 8 that developed parturient paresis, are shown in FIGURE 1.

It is evident that blood serum citric acid levels dropped at the time of parturition in both normally-calving and milk-fever cows, but the drop was more

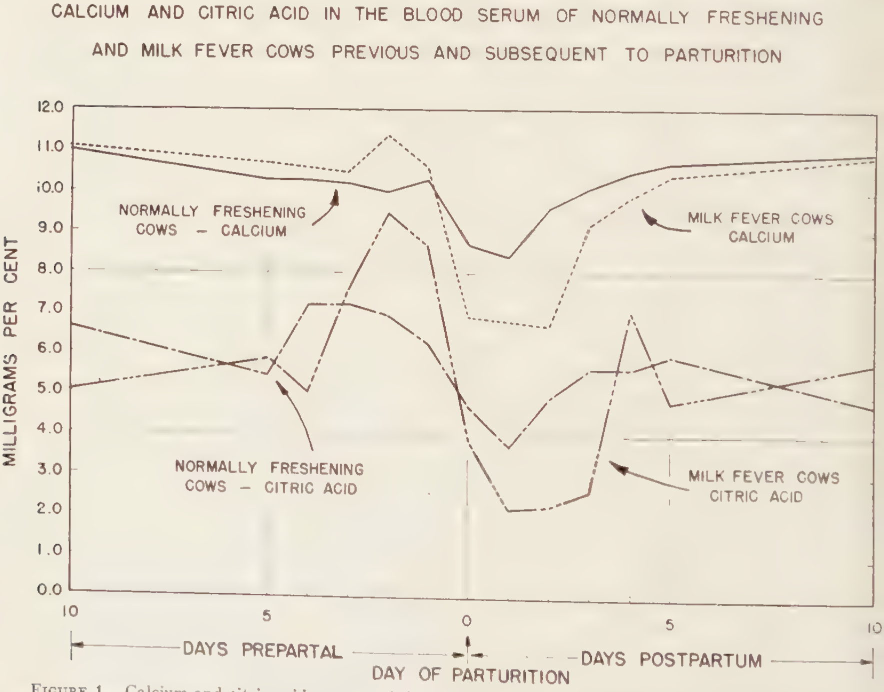

FIGURE 1. Calcium and citric acid content of the blood serum of normally freshening and milk-fever cows previous and subsequent to parturition.[6]

marked in the latter. Subsequent studies[8, 12] have confirmed these observations and have also demonstrated a significant correlation between blood serum citric acid levels and urinary citric acid excretion. From the limited data available to date, it is difficult to state whether the changes in citric acid levels of the blood are basic to, or incidental to, parturient paresis in dairy cows.

A Detailed Study of Levels of Several Blood Constituents Near the Time of Parturition

Ample data are available showing blood levels of various constituents at parturition in normally-calving cows and in cows with parturient paresis. There is a marked paucity of information, however, on the patterns followed by these constituents prior to the development of this disorder. In order to secure more information about the patterns followed by the several blood constituents known to be different in normally-calving and milk-fever cows, Van Soest and Blosser[38] initiated a study of this matter.

Eight cows were chosen, 4 with histories of milk fever at previous parturitions. In addition to drawing blood samples at daily intervals for several days prepartal and postpartum, samples were drawn every 4 hours from approximately 12 hours prepartal to parturition, every 3 hours for the first 24 hours postpartum, and every 6 hours for the second 24-hour period postpartum. Three cases of milk fever occurred in this study and 2 cows were considered borderline cases. These borderline cows exhibited blood symptoms typical of milk fever but did not become recumbent and unable to rise, and they required no treatment.

Blood serum calcium, blood glucose, phosphorus, pyruvic acid, and hematocrit were studied. FIGURE 2 shows graphically the blood serum calcium levels of the normally-calving, borderline, and milk-fever cows.

This graph shows the very erratic blood picture near the time of parturition, particularly in cows that developed milk fever. Very detailed information was obtained on one cow, 2116A. This cow appeared to be developing parturient paresis even before parturition. From 2 hours prepartal until 9 hours postpartum, during which time blood samples were drawn every hour, blood serum calcium values were 4.3, 4.7, 7.4, 7.8, 4.7, 6.4, 3.5, 5.0, 6.2, 6.4, 6.4, and 7.9 mg. per cent. By 9 hours postpartum the condition of this cow was improved, but by 24 hours postpartum she again appeared to be incoordinated. Blood samples drawn at 28, 31, 33.5, 34.5, 36.0, 37.0, and 39.5 hours postpartum showed blood serum calcium values at 2.6, 2.6, 6.7, 6.1, 3.3, 10.5, and 3.3 mg. per cent. Udder inflation at 34.5 hours postpartum did not seem to improve the condition of this cow, and she was treated with a calcium borogluconate solution at 39.5 hours postpartum. Data from other cows showed similar trends. Apparently in the conflict between factors tending to cause milk fever and compensatory mechanisms tending to prevent it, extremely erratic levels of blood constituents result.

Correlations between certain blood constituents when between-cow differences were removed showed highly significant correlations ($P < .01$) between glucose and pyruvic acid (+0.47), glucose and phosphorus (−0.84), and phos-

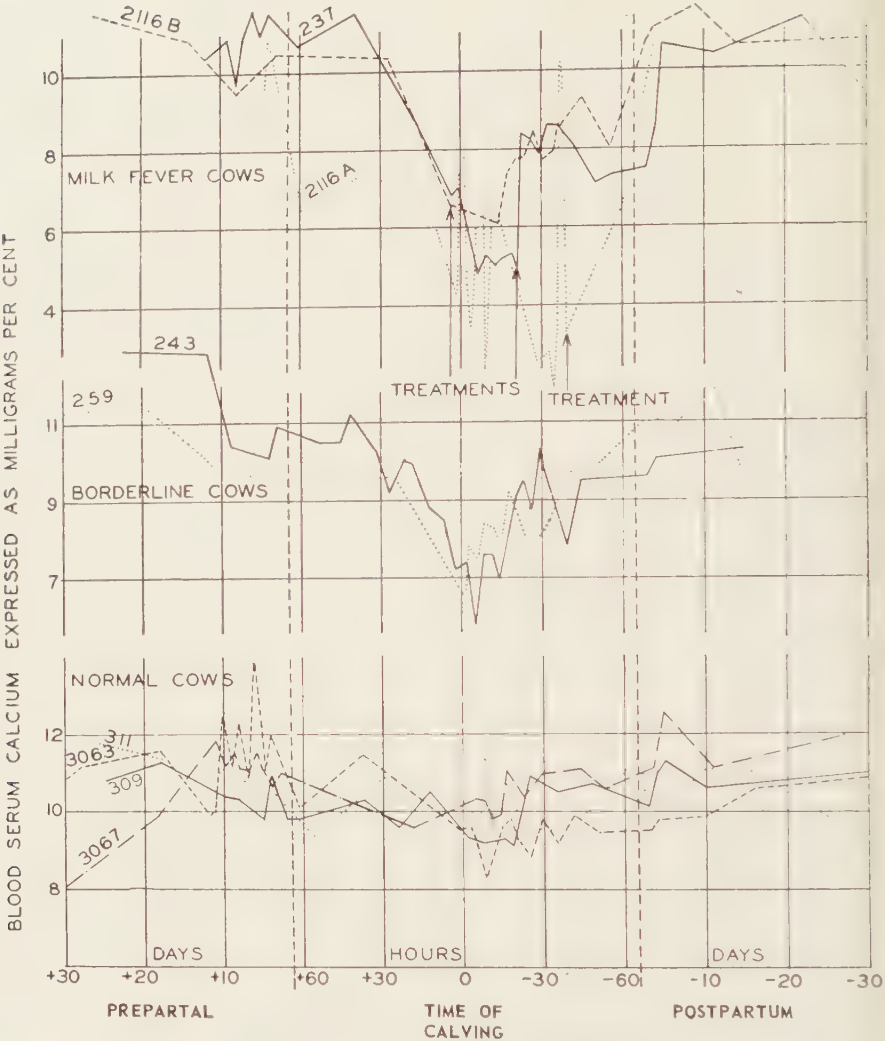

FIGURE 2. Blood serum calcium levels in normal, borderline, and milk-fever cows during the period near parturition.[38]

phorus and pyruvic acid (−0.59), both in the milk-fever and borderline cows. On the other hand, in normally-calving cows, correlations between the above constituents were all positive and only 1 (glucose versus pyruvic acid) was significant ($P < .05$).

Recent unpublished data[34] prove an interesting corollary to the experiments of Van Soest and Blosser.[38] Studying neutral steroid excretion of parturient cows, Soni[34] has shown a very erratic urinary excretion of these compounds during the development of attacks of parturient paresis. Excretion decreases erratically from about 24 hours prepartal until the time that the cow becomes recumbent, at which time excretion in some cases was so small as to be not measurable.

Urinary Excretion of Calcium and Other Minerals Near Parturition

When two dairy cows of equal lactating ability calve under the same herd-management conditions, one may develop parturient paresis and the other may not. This type of occurrence emphasizes the importance of seeking for causes of parturient paresis other than the initiation of lactation. While urinary channels are not as important quantitatively as are fecal channels in the elimination of many minerals, nevertheless, urinary studies are frequently used in studying mineral metabolism. Furthermore, parturient paresis is an abnormal state, and it is possible that abnormal kidney function might be related to this disorder. Little information is available on urinary excretion of minerals in dairy cows at parturition.

An investigation of the possible relation between changes in urinary excretion at parturition and changes in the blood picture was recently completed.[7] Of the 8 cows used in this experiment, 4 developed parturient paresis, 2 were borderline cases of this disorder, and 2 calved normally. Twenty-four-hour urine collections were made in a period starting about 30 days prior to the expected date of parturition and extending to 30 days postpartum. Blood samples were drawn at corresponding times.

In this trial urinary calcium excretion was quite variable at parturition. There was no consistent or appreciable variation between the normal, milk-fever, and borderline cows. Neither did a relation exist between calcium losses via urinary channels and changes in the blood serum calcium. From 16 to 3 days prepartal, magnesium was excreted in larger amounts in the urine of cows that subsequently developed parturient paresis than in the urine of normally-calving cows. Extremely small amounts of inorganic phosphorus were excreted via urinary channels by all except the 2 borderline cows on the second day following parturition. These 2 borderline cows excreted 10 times as much inorganic phosphorus on this day as any cows of the other groups at any period postpartum.

Ward *et al.*[39, 40, 41] have shown recently that cows subsequently developing parturient paresis are in a negative calcium balance for a month prior to parturition. More information is needed to demonstrate the reasons for this phenomenon.

Blood Levels and Urinary Excretion of Calcium in Parturient Cows Following Injection with Calcium Borogluconate

Following the classic investigations of Little and Wright,[22, 23] demonstrating that a hypocalcemia exists in parturient paresis, various types of calcium solutions were used in treating this disorder. Calcium gluconate appeared to be the material of choice because it was less antagonistic to tissues than others used. It had the discouraging feature, however, of being unstable in aqueous solutions. Hayden[17] and Austin[1] found methods of stabilizing and increasing the calcium content of calcium gluconate solutions by adding boric acid.

Since that time, 20 and 25 per cent solutions of calcium borogluconate have been widely used in treating milk fever.

Recently Austin[5] developed methods of preparing somewhat more concentrated solutions up to 32.7 per cent calcium borogluconate. With normal cows, Austin[5] found this material to be more effective than a 25.5 per cent solution in maintaining higher blood calcium values over a 24-hour period.

A study recently completed by Albright and Blosser[1,2] compared the effectiveness of the 25.5 per cent and the 32.7 per cent calcium borogluconate solutions in treating milk fever. In addition, urinary calcium excretion in normal parturient cows and in cows developing parturient paresis was studied. This study involved 14 cows developing parturient paresis, 5 normally-calving multiparous cows, and 3 normally-calving primiparous cows. Eight of the 14 cases of parturient paresis were treated with the 32.7 per cent solution and 6 with the 25.5 per cent solution. The calcium borogluconate was injected at the rate of 0.5 ml. lb. body weight. Blood samples were taken from all cows daily for 3 days prior to the expected date of parturition, at 15 minutes postinjection, every 2 hours for 24 hours subsequent to injection, and at 36, 48, and 72 hours postinjection.

The procedure for injection of calcium borogluconate solutions into normal parturient cows was essentially the same except that these cows were injected intravenously with 1 of the 2 calcium solutions at 12 hours post-calving if they did not exhibit milk-fever symptoms by this time. Most dairy cows that eventually develop milk fever begin to show symptoms by 12 hours postpartum.

Urine collections were made every 2 hours by the method described by Turner *et al.*[37]

Following treatment with the 32.7 per cent solution, 4 of 8 cows relapsed into a second attack of milk fever, while 3 of 6 treated with the 25.5 per cent solution relapsed. On this basis, noting the blood serum calcium values, there would appear to be no advantage in treating milk-fever cows with the more highly concentrated solution. While the 32.7 per cent solution caused a higher initial rise in blood serum calcium levels, by 6 to 10 hours postinjection blood serum calcium levels were essentially the same and remained so for the remainder of the 24 hour sampling period. Likewise, urinary excretion of calcium in the two groups was nearly the same by 8 to 10 hours postinjection.

In comparing the response to injection of calcium borogluconate solutions in milk-fever and normal cows, it is evident that normal cows responded with a somewhat higher blood serum calcium level immediately following injection. This response was expected since blood serum calcium levels were higher at the time of injection in the normal parturient cows. There was no evidence from these data that milk-fever cows behave differently from normally-calving cows in response to injection of calcium borogluconate solutions.

Several interesting sidelights developed as a result of this study. Two of 3 normal parturient cows injected with the 32.7 per cent solution developed parturient paresis 47 hours after calving (35 hours postinjection) even though they exhibited no symptoms and had normal blood calcium levels 12 hours postpartum. Neither of the 2 cows injected with the 25.5 per cent solution developed this disorder. While the occurrence of the first attack of milk fever following parturition as late as 47 hours postpartum is not unheard of, it is uncommon. Conclusions cannot be drawn from so few data, but it would appear that the injection of calcium solutions at parturition, as sometimes recommended to prevent milk fever, is not only ineffective in warding off this disorder but, in large amounts, may even precipitate milk-fever attacks.

A further indication of possible depressing effects of injected calcium on calcium metabolism in normal parturient cows was evidenced in one of the three primiparous cows injected with the 32.7 per cent solution. This cow, injected 12 hours postpartum, had a blood serum calcium level of 7.4 mg. per cent at 24 hours postinjection and appeared slightly incoordinated at this time but was not treated. This animal exhibited normal blood serum calcium values by 36 hours postinjection. Primiparous cows do not ordinarily show a decline in blood serum calcium to this level and seldom if ever develop milk fever.

Summary

During the past 10 years considerable progress has been made in research on parturient paresis. Certain commonly recommended preventive measures have been shown to be ineffective while, on the other hand, effective means of prevention have been found. Mastectomy has shown that the initiation of lactation at parturition is important in the development of parturient paresis, but factors other than lactation play key roles in the development of this disorder. Rather detailed studies have been made on blood levels of calcium and other inorganic and organic constituents following treatment of parturient paresis by udder inflation and following injection of calcium borogluconate solutions. Important in the interpretation of this research are studies on the behavior of normal parturient cows under the same conditions. Patterns followed by certain blood constituents prior to parturition have also been established by frequent collection of blood samples prior to parturition and the development of parturient paresis. Finally, mineral balance studies have been conducted showing, for the first time, mineral balances immediately prior and subsequent to parturition in normally-calving and milk-fever cows.

The blood and urinary excretion picture in parturient paresis of dairy cows does not conform to that of hypoparathyroidism in man and laboratory animals. Hypoparathyroidism is characterized by a hypocalcemia, a hyperphosphatemia, and by decreased renal elimination of calcium and phosphorus. Parturient paresis, on the other hand, is characterized by a hypocalcemia, a hypophosphatemia, and no change in the renal elimination of calcium and phosphorus.

Even with our increased knowledge of parturient paresis, many aspects of this disorder remain unexplained. If one could explain why cows of certain breeds are more susceptible than cows of other breeds, why primiparous cows seldom if ever develop the disorder, and why certain individual cows seem more prone to develop milk fever than other cows of equal age and producing ability, then the etiology of this disorder could be more adequately explained.

References

1. Albright, J. L. 1954. The influence of calcium borogluconate on the blood levels and urinary excretion of calcium in dairy cows with parturient paresis. M. S. Thesis, State College of Washington, Pullman, Wash.
2. Albright, J. L. & T. H. Blosser. 1954. The influence of intravenously injected calcium borogluconate on the blood levels and urinary excretion of calcium in dairy cows with parturient paresis. J. Dairy Sci. **37:** 661.
3. Alwall, N. 1944. Studien über die Regulation vom Citronensauergehalt des Blutserums, ein Beitrag ner Frage des Zusammenhangs zwischen Calcium und Citronensauer. Acta Med. Scand. **116:** 337.
4. Austin, J. A. 1935. Process for stabilization of calcium gluconate solutions. Patent No. 2,007,786. Off. Gaz. U. S. Pat. Off. **456:** 449.
5. Austin, J. A. 1952. Personal communication.
6. Blosser, T. H. & V. R. Smith. 1950. Parturient paresis V. Blood serum levels of citric acid and calcium in normal parturient cows and cows with parturient paresis. J. Dairy Sci. **33:** 81.
7. Blosser, T. H. & V. R. Smith. 1950. Parturient paresis VI. Some changes in the urinary excretion of certain constituents at parturition and their possible association with changes in the blood picture. J. Dairy Sci. **33:** 329.
8. Blosser, T. H. & G. M. Ward. 1954. Blood levels and urinary excretion of citric acid at parturition. Proc. 35th Ann. Meet. West. Div. Am. Dairy Sci. Assoc.
9. Boda, J. M. & H. H. Cole. 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever in dairy cattle. J. Dairy Sci. **37:** 360.
10. Boddie, G. F. 1949. Metabolic disturbances in ruminants. Vet. Rec. **61:** 85.
11. Dickens, F. 1941. The citric acid content of animal tissues with reference to its occurrence in bone and tumour. Biochem. J. **35:** 1011.
12. Dryerre, H. & J. R. Greig. 1925. Milk fever: its possible association with derangements in the internal secretions. Vet. Record. **5:** 225.
13. Dryerre, H. & J. R. Greig. 1928. Further studies in the etiology of milk fever. Dumfries and Galloway Vet. Med. Assoc. Oliver and Boyd. London, England.
14. Duckworth, J. & G. M. Ellinger. 1950. Ovarian hormones and calcium metabolism. J. Endocrinol. **7:** 7. (Chem. Abstr. **45:** 3053. 1951).
15. Freeman, S. & T. S. Chang. 1950. Effect of thyroparathyroidectomy and vitamin D upon serum calcium and citric acid of normal and nephrectomized dogs. Am. J. Physiol. **160:** 341.
16. Greig, J. R. 1926. Milk fever. Vet. Record. **6:** 625.
17. Hayden, C. E. 1934. A method for making concentrated calcium gluconate solutions. Cornell Vet. **24:** 93.
18. Hebert, F. K. 1952. Hyperparathyroidism. Diagnostic difficulties and complications. Inter. Congress Biochem. Abstr. of Commun. 1st Congress, Cambridge, England (1949). 166. Chem. Abstr. **46:** 8247.
19. Hibbs, J. W. 1950. Milk fever (parturient paresis) in dairy cows—a review. J. Dairy Sci. **33:** 758.
20. Hibbs, J. W. & W. D. Pounden. 1955. Studies on milk fever in dairy cows IV. Prevention by short-time prepartal feeding of massive doses of vitamin D. J. Dairy Sci. **38:** 65.
21. Klotz, H. P., P. Barbier, R. Miranda & B. Cantorovich. 1949. Effect of estrogens on blood calcium of normal and parathyroidectomized dogs. Ann. endocrinol. (Paris). **10:** 445 (Chem. Abstr. **46:** 10335. 1952)
22. Little, W. L. & N. C. Wright. 1925. The etiology of milk fever in cattle. Brit. J. Exptl. Pathol. **6:** 129.
23. Little, W. L. & N. C. Wright. 1926. A review of some modern theories on milk fever. Vet. J. **82:** 185.
24. Nicolayson, R. & R. Nordb. 1943. Calcium metabolism and citric acid. Acta Physiol. Scand. **5:** 212. (Chem. Abstr. **38:** 5268. 1944).
25. Niedermeier, R. P. & V. R. Smith. 1948. A study of some blood constituents of cows not milked following parturition. J. Dairy Sci. **31:** 684.
26. Niedermeier, R. P. & V. R. Smith. 1950. Parturient paresis IV. The effect of udder inflation upon blood levels of calcium, magnesium and phosphorus in cows with parturient paresis. J. Dairy Sci. **33:** 38.
27. Niedermeier, R. P., V. R. Smith & C. K. Whitehair. 1949. Parturient paresis III. A study of various blood constituents at parturition in mastectomized cows. J. Dairy Sci. **32:** 927.

28. Perkins, H. R. & J. T. Dixon. 1953. Parathyroid and bone citrogenase. Science. **118:** 139–140.
29. Pincus, J. B., S. Natelson & J. K. Lugovoy. 1951. Effect of adrenalin, adrenocorticotropic hormone (ACTH), and cortisone on citrate, calcium, glucose, and phosphate levels in rabbits. Proc. Soc. Exptl. Biol. Med. **78:** 24.
30. Shorr, E., T. P. Almy, M. H. Sloan, H. H. Taussky & V. Toscani. 1942. The relation between the urinary excretion of citric acid and calcium; its implications for urinary calcium store formation. Science. **96:** 587.
31. Shorr, E., A. R. Bernheim & H. H. Taussky. 1942. The relation of urinary citric acid excretion to the menstrual cycle and the steroidal reproductive hormones. Science. **95:** 506.
32. Smith, V. R. & T. H. Blosser. 1947. Parturient paresis I. The incidence of parturient paresis and changes in the total blood serum calcium at parturition in prepartal milked cows. J. Dairy Sci. **30:** 861.
33. Smith, V. R. & R. P. Niedermeier. 1948. Parturient paresis II. The effect of partial versus complete milking upon the total blood serum calcium of dairy cows at parturition. J. Dairy Sci. **31:** 173.
34. Soni, B. K. 1954. A study of the excretion of certain steroid hormones at the time of parturition in normal cows and in cows affected with parturient paresis. Ph.D. Thesis, State College of Washington. Pullman, Washington.
35. Steenbeck, H. & S. A. Bellin. 1953. Vitamin D and tissue citrate. J. Biol. Chem. **205:** 985.
36. Turner, C. W. 1931. The development of the mammary gland as indicated by the initiation and increase in the yield of secretion. Mo. Agr. Exp. Sta. Res. Bull. 156.
37. Turner, C. W., A. H. Frank, C. H. Lomas & C. W. Nibler. 1930. A study of the estrus producing hormone in the urine of cattle during pregnancy. Mo. Agr. Exp. Sta. Bull. 150.
38. Van Soest, P. J. & T. H. Blosser. 1954. A detailed study of levels of certain blood constituents in normally calving dairy cows and in dairy cows with parturient paresis. J. Dairy Sci. **37:** 185.
39. Ward, G. M., T. H. Blosser & M. F. Adams. 1952. The relation of prepartal and postpartum mineral balances to the occurrence of parturient paresis in dairy cows. J. Dairy Sci. **35:** 587.
40. Ward, G. M., T. H. Blosser & M. F. Adams. 1953. Mineral balances at parturition as related to the occurrence of parturient paresis in dairy cows. Wash. Agr. Exp. Sta. Tech. Bull. No. 5.
41. Ward, G. M., T. H. Blosser & M. F. Adams. 1953. The intake and excretion of minerals at parturition as related to the occurrence of parturient paresis in dairy cows. Wash. Agr. Exp. Sta. Circ. 220.
42. Ward, G. M., T. H. Blosser, M. F. Adams & J. B. Crilly. 1953. Blood levels of some inorganic and organic constituents in normal parturient cows and cows with parturient paresis. J. Dairy Sci. **36:** 39.

Part V. Special Aspects of Calcium Metabolism

SOME OBSERVATIONS ON RICKETS AND ALLIED BONE DISEASES IN SOUTH AFRICAN DOMESTIC ANIMALS

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Introduction

When it was originally suggested that I should contribute to this discussion on disorders of calcium and phosphorus metabolism, I was immediately advised in no uncertain terms by a colleague who is familiar with the American scene, to discuss anything I pleased but to avoid the subject of rickets. Before I begin, therefore, I must plead that I placed my credentials before the organizers of the conference on which this monograph is based, who nevertheless replied that they were confident that what I had to offer would be relevant and acceptable. Even so I shall make my contribution with some trepidation.

My approach is clinical and practical rather than chemical and theoretical, and I must state from the outset that when I make some blatantly heretical statements I do so with the consciousness that these may quite possibly apply only to our own conditions in South Africa. One thing we have a lot of, and to spare, is the sun, and as the sun has a great deal to do with the synthesis of vitamin D our conditions cannot very easily be compared with those of much of the North American continent or of Europe. But we do get rickets in our dogs and sheep and osteomalacia in our cattle. It is about these that I want to offer some remarks, together with some observations on osteodystrophia fibrosa in the various animals, and on the interrelationships with each other.

Etiology

One feels sorry for a student who has to consult the available textbooks and hopes to gain from them a clear impression of what causes rickets. Fundamentally one must accept the fact that there is, for some reason, an inadequate deposition of minerals in the osteoid matrix of immature bone. Osteomalacia is a similar condition occurring in adult animals under circumstances of dietary mineral deficiencies. According to Theiler (1934) rickets is a disease of foals, calves, lambs, kids, young pigs, and puppies. Osteomalacia is a disease of horses, cows, sheep, and goats, and, less often, of pigs and dogs. Theiler emphasizes that the variations in the pathological picture of rickets can be attributed to differences in intensity of growth, external influences, and mechanical factors. Animals reaching sexual maturity and being used for breeding long before their growth has ceased, when their bodies have to meet the demands first of pregnancy and then of lactation in addition to those of growth, are particularly vulnerable to rickets.

Calves are never afflicted with rickets at birth, but they easily develop the condition on phosphorus-deficient pastures. In pigs, Theiler makes the interesting observation (interesting in view of remarks to be made later) that rickets occurs alone or with the complication of osteodystrophia fibrosa *under dietary conditions differing very little from those causing pure rachitic lesions.* Theiler accepted the fact that true rickets occurred in dogs, but also osteodystrophia fibrosa, a *disease that may be clinically mistaken for rickets.* After examining the available evidence Theiler concluded that with an inadequate supply of vitamin D the relative amounts of calcium and phosphorus were of greater importance than their absolute amounts. With sufficient vitamin D there was much more latitude in imbalance of the minerals and, similarly, if the minerals were present in the correct proportion, the vitamin D requirements were very low. In his final summing up he regarded rickets in the dog to be, for practical purposes, a vitamin-D-deficiency disease.

In his experiments with heifers in abundant sunlight, thus with abundant vitamin D, he could produce rickets and osteomalacia only with low-phosphorus rations and not with a calcium-deficient diet. In young cattle he therefore regarded rickets as a pure phosphorus-deficiency disease. Under experimental conditions the same observations were found to apply to sheep. The condition of "stiff-sickness," that is, rickets and osteomalacia of cattle, is accompanied by intense bone hunger and, under natural conditions of phosphorus deficiency, cattle pick up bones and pieces of carcasses of any dead animals and, by so doing, ingest sufficient quantities of *Clostridium botulinum* toxin to die of botulism. Until this chain of events was understood, cattle farming could not be profitably carried out over large tracts of country later found to be phosphorus deficient.

In respect to pigs, Theiler's conclusion was that the deficiency was usually calcium, there being a sufficiency of phosphorus in their concentrate rations.

Theiler, furthermore, made an extensive pathological study of Recklinghausen's disease, or osteodystrophia fibrosa, in horses, donkeys, and mules, since in South Africa under all ordinary conditions this disorder is the usual manifestation of "bone disease." Horses under 7 years of age were most susceptible, as were horses doing heavy work, and race horses in training. Quite obviously the disease was associated with fast growth or heavy physical demands. The malady was variously referred to as "bran disease" or "miller's disease," because of the presumed etiological significance of this item of diet, and "big head," because of the enlargement brought about by the development of large amounts of fibrotic, tumorlike tissue in the maxillae. Just before the turn of the century there were severe "outbreaks" of the malady in racing stables and in military remount camps. Many puzzling features of these outbreaks sometimes suggested hereditary causes, but mostly the transmission of an infective agent was indicated. In the course of time, when incidence decreased and the disease appeared only sporadically, it became clear that the cause was simply an unbalanced diet characterized by low calcium with excess of phosphorus. Many of the lamenesses, spavin, splints, and ringbones of horses were explained on a basis of osteofibrosis, even if jaw and maxillary swelling were not yet evident.

Subsequently Groenewald (1949), apparently feeling that the matter was not so simple, conducted some feeding trials on six-month-old fillies and colts, giving calcium at various levels but with a marked excess of phosphorus. In a period of about a year these animals evidenced osteofibrosis, osteoporosis *and* rickets. Groenewald found that there were a number of factors influencing the absorption and utilization of calcium and phosphorus and that, under conditions of excess of phosphorus, different histopathological pictures could be produced. The age of the animals at the start of these experiments seemed to have some influence on the final pathological picture.

In a small group of goats, Groenewald and his co-workers (1940) experimentally produced osteodystrophic diseases, both osteofibrosis and rickets.

In nature, however, the unproductive goats of South Africa, generally accustomed to fend for themselves on poor pasturage, seldom show these conditions.

Thomas, in 1928, recorded the incidence of "bent leg" in sheep in eastern Transvaal, where the mothers and lambs were fed wheaten bran and crushed maize in supplement of their ration of cultivated and natural pastures. This diet made a mineral imbalance likely, but at the time of the study the disease had healed, although the deformity was still present. An interesting observation was that the fastest growing lambs were the ones to show clinical symptoms. Since that time, this type of bone involvement, on a number of occasions, has been observed in South Africa, notably in ram lambs fed on green wheat, barley, or oats for the greater part of the day, starting from the time of weaning. Reinach and Adelaar (1955) ascribed this condition to a combination of (1) fast growth; (2) a specific rickets "conditioning" factor in green cereals as found by Ewer (1950) and others which specifically interfere with the uptake of phosphorus; and (3) the rachitogenicity of androgens as shown by Norman and Mittler (1948) in experiments with rats.

After consideration of all these findings in past years in South Africa the conclusion is inescapable that, while rickets and osteodystrophia fibrosa are histopathologically distinct entities, the differentiation is, clinically and from the practical point of view, not so distinct. Even the etiology is not as easily separated as was first thought. My own impression has been, certainly with dogs, that pathological examination of biopsy specimens of costochondral junctions of undoubted clinical and radiological rickets cases sometimes reveals rickets and sometimes osteofibrosis.

More than those of mineral deficiencies, there are very important considerations in the causation of rickets (in the clinical and radiological sense) in dogs under conditions peculiar to South Africa. These include:

(1) Very rapid growth, brought about by an overenthusiastic, overliberal, "forcing" type of diet, that may contain excessive cereals, fat, yeast, eggs, milk, wheat germ oil, and pills containing supplements of minerals and vitamins.

(2) The feeding of cod- or other fish-liver oils, inevitably in a more or less rancid state, and always in excessive quantities.

(3) The incidence of enteritis, and particularly coccidiotic or verminotic enteritis, at a period of rapid growth, resulting in poor assimilation of food elements. The regular administration of liquid paraffin has the same effect.

A number of instances of rickets observed at Onderstepoort and illustrating some of these contentions, now follow.

Police Dogs

Clark, Thomas, and Schulz (1941) described their experiences with one of the offspring of a Rhodesian Ridgeback dog belonging to the South African Police. Litters from 3 bitches had included rickety pups, and the common factor was the sire. A normal pup from 1 of these litters, was mated, in due course, to some nondescript bitches and, of a number of normal pups, one was born showing rickets, and was studied. The siblings grew well and presented a marked contrast. The investigators considered that a hereditary factor in the etiology was a possibility to be investigated.

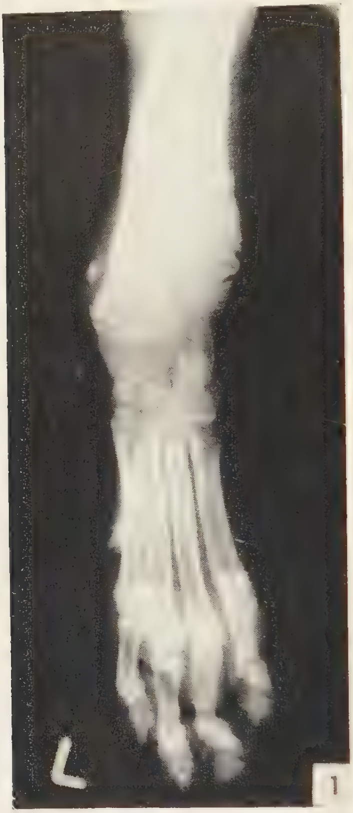
Some years later (1951) there was another complaint of a weak litter of 10, of which 5 died immediately from weakness. The remaining 5 were presented for study. These were under 3 months old, of good size, but with arched backs, and rather poorly fleshed. Two were bowlegged in front, and the joints were enlarged though not painfully. The costochondral junctions were all enlarged, and the whole litter had a more or less rickety appearance. The diet had consisted of mothers' milk supplemented by 1 lb. of maize meal, 1 oz. of kaffir corn malt (sprouted sorghum), and 1 pt. of milk daily. This was estimated to provide unbalanced calcium and phosphorus figures of 1 gm. and 3.9 gms. respectively, and insufficient vitamin A. Rib biopsies done at this time showed the classical picture of osteodystrophia fibrosa (Schulz, 1952). For three months the diet was supplemented by 5 per cent meat meal (for 2 pups) and 15 per cent fresh meat (for the other 2) and biopsies of costochondral junctions after this period (Clark, 1952) showed practically complete healing of the osteodystrophic condition. The fifth pup had died shortly after admission. It was concluded that pups may grow fairly well on an unbalanced diet and yet be suffering from very severe bone disease, and that the incidence of such a condition in a litter of pups is not necessarily due to any hereditary trait.

Since this period, we have from time to time encountered cases of bone disease in dogs from homes where the cost of feeding was no object, and where no effort was spared to give the animal the best possible care.

Some Case Reports

Case 1. Marco, a boxer dog 7 months old, had cost a lot of money, and the owners lavished every possible care on him, including a daily dose of cod liver oil, lavish vitamin supplements, and extra minerals. His food was of excellent quality. For three or four weeks Marco had been ill with irregular fevers (up to 105° F.) and continuous pain centering on the joints of his legs. He was lame and decidedly uneasy on his feet. Strangely, his appetite remained unimpaired. All sorts of treatment had been applied, more vitamins, antibiotics, salicylates, and antihistamines.

On examination the blood picture was found to be normal, with an erythrocyte sedimentation rate of 0 mm. in 1 hour (Wintrobe tubes) and a complete absence of bile pigments. The leukocytic differential count was also normal. Urine examination was negative in all respects and the feces contained no helminth ova. X-ray examination showed decidedly disordered epiphyseal lines with "lipping" of the edges and separation of the epiphyses from the



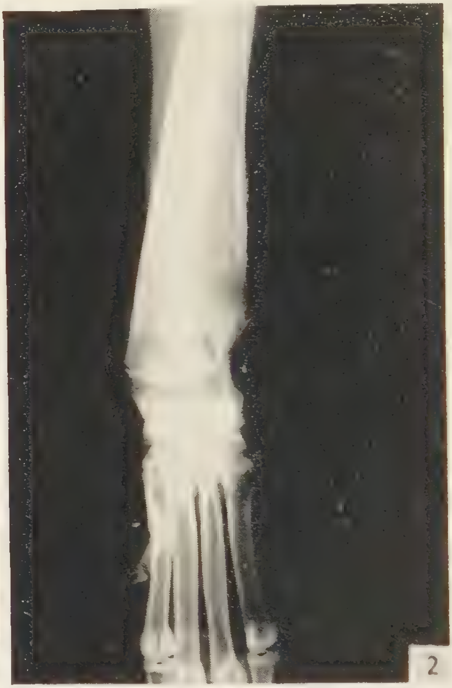


Figure 1. Radiograph of carpal and metacarpal region of Case 1 on admission. Rickets.
Figure 2. Carpus and metacarpus of Case 1 after 6 weeks' dietary adjustment.

diaphyses. The criteria laid down by Schnelle (1950) were clearly applicable, and spicules of bone were to be seen all around the thickened and moderately distorted joints. Detachment of the olecranon was quite evident in the elbows. Some of these lesions can be seen in FIGURE 1.

The diagnosis of rickets was very coolly received by the owners.

The dog was placed on a simple diet of raw meat, corn-meal porridge, and milk, with a supplement of 2 tablespoons daily of a mixture of 400 gm. casein, 25 gm. torula yeast, 10 gm. mineral mixture, and 1 gm. Rovimix A (50,000 IU vitamin A per gm.). The mineral mixture consisted of 300 parts dicalcium phosphate, 150 parts calcium lactate, 150 parts calcium carbonate, and 15 parts ferric ammonium citrate.

The response was immediate. Fever was no longer in evidence and, within a few days, the dog moved much more easily. No drug treatment was given. After 6 weeks the radiograph depicted in FIGURE 2 was taken. The lesions had disappeared and the legs were straight. The animal was discharged.

Case 2. At about the same time another boxer pup called Allderman was referred to Onderstepoort with a diagnosis of Marie's disease (pulmonary osteo-

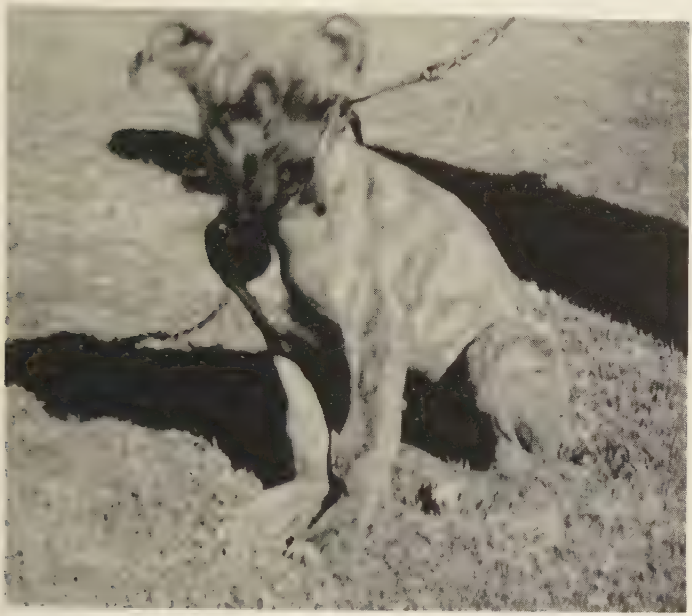


FIGURE 3. Boxer pup "Allderman" showing rickets. Case 2.

arthropathy). There was very marked deformity and thickening of the joints, with severe lameness. Allderman was five to six months old, but not very well grown. Large quantities of codliver oil had been fed to the pup, in addition to an excellent diet by all ordinary standards. The deformity and thickening of the forelegs are shown in FIGURE 3, but the "cow-hocked" appearance of the hindlegs is not so evident.

Apart from the bone condition, the clinical examination was essentially negative except that there was a mild *Ascaris* infestation that was immediately eliminated. In FIGURE 4 the radiological picture is illustrated. This shows the error of the original diagnosis and reveals the typical picture of rickets.

Allderman was placed on a diet similar to that used in case 1, but, as his was such a bad case, it took somewhat longer to clear up, and a certain amount of deformity persisted after the bone tissue had become quite normal. The radiographic examination (FIGURE 5) shows the forelegs when the lesions were entirely healed. It should be mentioned here that, while this animal presented radiologically and clinically a good case of rickets, the pathological report on a rib biopsy shortly after admission gave a pathological diagnosis of osteodystrophia fibrosa.

Case 3. This animal, a very well-grown 6-months-old Alsatian (German shepherd) called Paddy, was presented showing deformity and lameness, with decided thickening of the joints. This animal was the pet of an elderly lady who lavished every possible care and attention on him and was most horrified to observe the increasing lameness. Cod liver oil could again be incriminated. The response to the conservative diet was very rapid. Only one month sep-

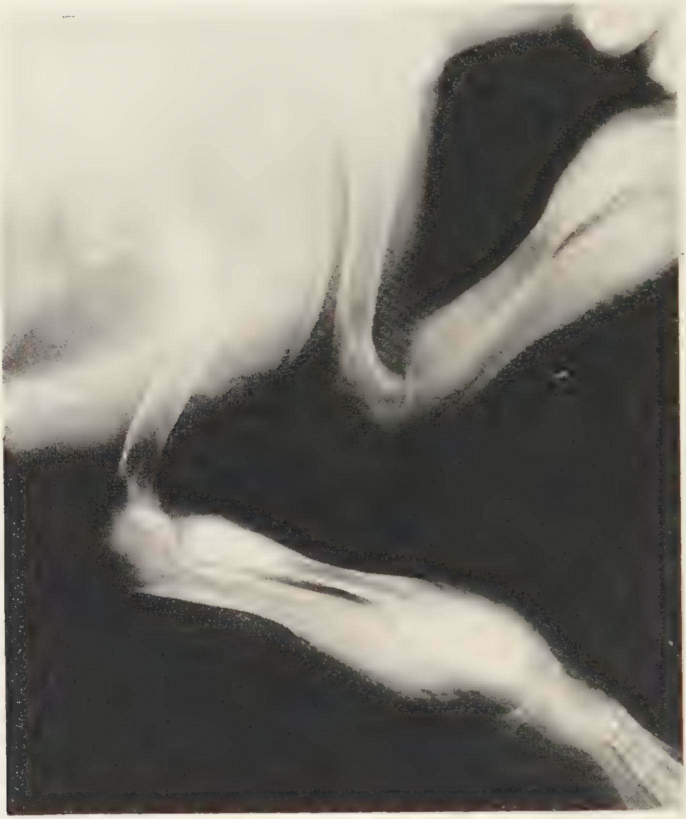


FIGURE 4. X-ray appearance of Case 2 on admission. Advanced rickets.

arated the radiograph in FIGURE 6 from that depicted in FIGURE 7. The carpus was practically straight on discharge.

Therapy

The investigations of Onderstepoort workers originally headed by Theiler (Theiler, Du Toit, and Malan, 1937a; Theiler, Du Toit and Malan, 1937b.) have shed sufficient light on the incidence of bone disease in South Africa to make it clear that vitamin D can, for the most part, be disregarded, and that the absolute and relative quantities of calcium and phosphorus were of primary importance.

It thus became possible to farm cattle with profit in phosphorus-deficient pasture which, in other respects, was first class ranching country, merely by providing bone meal, bone flour, or dicalcium phosphate regularly. Horses developing bone diseases could be treated with calcium to cover their deficiencies, as could pigs. In the case of rickets of young weaner sheep, especially as occurring among ram lambs, it was found that bone meal or dicalcium phosphate or phosphate supplements in drinking water provided the answer, together with a limitation of grazing time on green winter cereals to 1 hour daily.

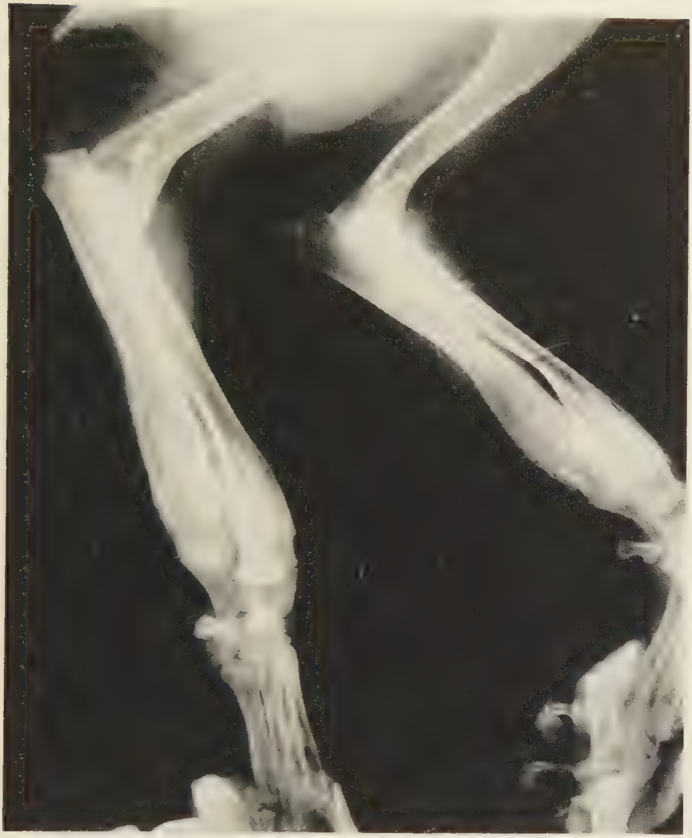


FIGURE 5. Case 2 about two months after admission.

The treatment of rickets, in view of the more "circumstantial" incidence of the disease, or perhaps the more artificial conditions of management, is decidedly less simple. Examining various textbooks, one is struck by the uneasiness displayed by most authors as regards categorical recommendations as to treatment. Mayer (1953), for instance, recommends the provision of calcium- and phosphorus-rich foods, but thinks that calcium supplementation might be safer as this element is more likely to be deficient. Then, since it is difficult to establish a really favorable ratio in practice, he advises vitamin dosage in amounts above those known to be effective. Quoting Morgan's (1940) experience that 250 IU per kilogram doses did not protect his cases, Mayer considers still higher dosage to be advisable, but immediately warns against the possible toxic effects of high dosage and goes on to say that combinations of vitamins A and D should be used rather than D alone.

Kirk (1954) states categorically that "rickets is due not so much to a calcium deficiency as to a lack of phosphate and of vitamin D," and that "no amount of calcium in the absence of sufficient vitamin D and of phosphorus will cure rickets."

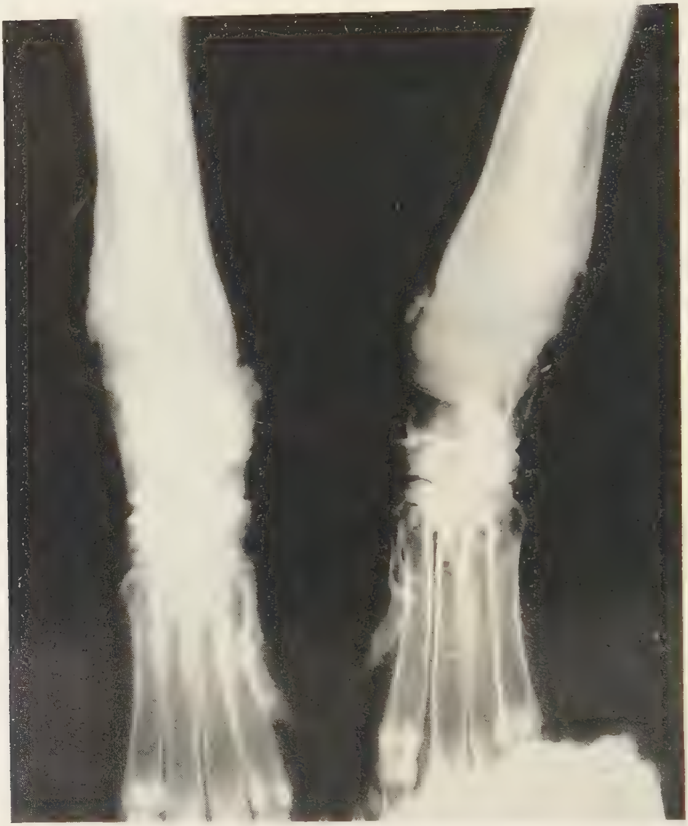


FIGURE 6. Radiograph of Case 3 on admission

In South Africa we have found that, for practical purposes, we can afford to ignore vitamin D requirements, as the sun is more than adequate over the greater part of the country most of the year. If any supplementation is needed, a stable source of vitamin A (Mellanby, 1947) and some of the vitamin B complex could be added in small quantities. All oils should be avoided and the diet should be simple, with the cereals somewhat restricted.

Any verminosis or coccidiosis should be dealt with immediately, particularly during the period of active growth. Any enteritis of bacterial or other origin should be dealt with expeditiously and, as a distemper infection easily precipitates rickets, adequate measures toward immunization against this disease should be taken at the age of about 3 months.

Discussion

From the assembled evidence on the occurrence of bone disease in horses, cattle, sheep, and pigs in South Africa, it is clear that the etiologies have been reduced to fairly simple deficiencies or imbalances of minerals, with vitamin

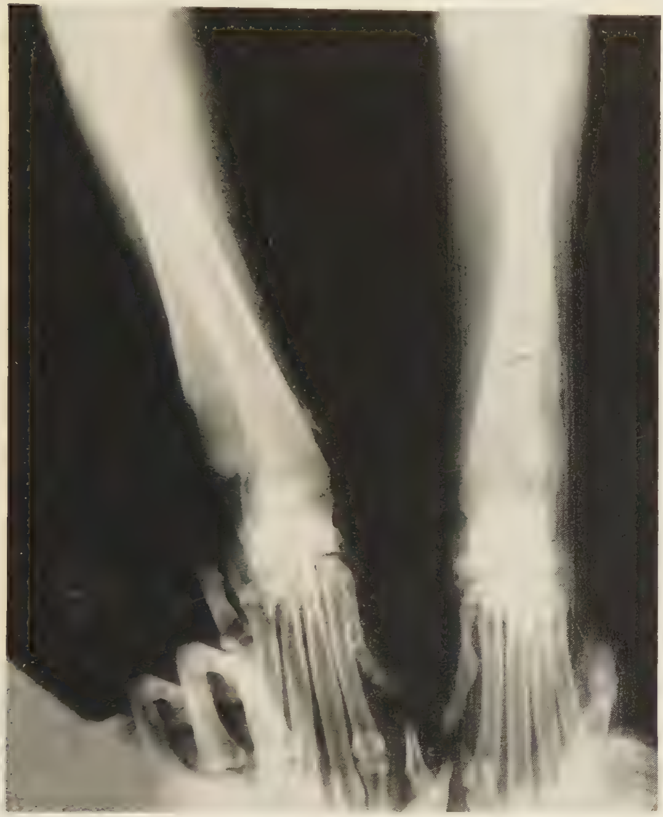


FIGURE 7. Radiograph of Case 3 one month after admission

deficiencies occupying a minor place. This situation would naturally not be directly or necessarily applicable to other countries with different climatic and pastural conditions.

New light, however, has been shed during the past 2 or 3 years on the causation of rickets in dogs under our conditions. Most prominently, fish-liver oils have emerged as an important factor in the aggravation, if not actually the promotion of delayed epiphyseal closure. The pathological identity of this lesion, incidentally, whether true rickets or osteodystrophia fibrosa, appears to me to be unimportant or, at best, of academic significance, for the reason that they are clinically or radiologically indistinguishable.

A glimpse at some of the overseas literature has given the impression that the deterioration of fish-liver oils, and the harm done by them, is experienced not only in our hot dry climate but also in places such as Canada, under vastly different conditions.

Swain and Idler (1955), for instance, in a recent report from the Pacific Fisheries Experimental Station, Vancouver, British Columbia, Canada, have described investigations into the factors that affect the stability of fish-liver oils, particularly in respect to vitamin A. Marked differences had been observed in the stability of different batches of oil, and these experiments were conducted in order to arrive at a means of producing stable oils. It was known that vitamin A is rapidly destroyed by rancidity, by moisture content, and by the presence of metal contamination. These workers found that if a stable and an unstable oil were mixed, the stability of the mixture was rapidly destroyed. In emulsion form, from 90 to 100 per cent of vitamin A was destroyed in three days. Iron contamination through rusty equipment also caused instability, which could be counteracted by the use of versene, a chelating agent.

Oxidation was an important factor in deterioration, one which in practice probably plays the most important role. In good oils an antioxidant (bytylated hydroxyanisole) was active in preventing loss of vitamin A in quantities below the permitted level (0.2 per cent). Some oils, however, were so unstable that no amount of BHA could prevent complete destruction.

These investigators then recommended the use of rust-free equipment, the addition of versene, rapid processing of oil (not leaving it for any length of time in an emulsified state), not mixing different batches of oil before determining stability, and the use of fresh livers only.

Swain and Idler's report has been discussed at some length in order to indicate the complexity of the whole question of the fish-liver oils and their stability. It helps one to understand the reasons for the failure of such oils under South African conditions.

Another factor that one should mention is the prevention of assimilation of fat-soluble vitamins by the regular presence of oil in the alimentary tract. This has been shown to be a factor of no mean significance. It thus seems to be no accident that muscular dystrophy, possibly induced as much by destruction or nonassimilation of vitamin E as by the avoidance of exercise because of painful joints, is a usual accompaniment of rickets. The induced deficiency of vitamin A will also have the usual effect of lowering resistance to disease and affections of the ectodermal tissues.

In my introduction I mentioned that my approach was to be clinical and practical. I am afraid that in some respects I have strayed, perhaps unavoidably, into theoretical paths, paths with which I am less familiar. For such aberrations I crave your indulgence.

In conclusion I wish to record my sincere appreciation of the enlightening discussions I have been able to have on these subjects from time to time with my colleague J. W. Groenewald, head of the Department of Nutrition at Onderstepoort.

References

Clark, R. 1952. Unpublished pathological report.

Clark, R., A. D. Thomas & K. C. A. Schulz. 1941. An unusual case of rickets in a dog. J. S. African Vet. Med. Assoc. **12:** 73–80.

Ewer, T. K. 1950. Rachitogenicity of green oats. Nature. **166:** 732–733.

Groenewald, J. W. 1949. Bone disease in equines. Rep. XIV. Intern. Vet. Congr. 1949. Sect. 4(a)(i).

Groenewald, J. W., A. D. Thomas & B. A. du Toit. 1940. Experimental osteodystrophic diseases in goats. Onderstepoort J. Vet. Sci. Animal Ind. **15:** 229–309.

Kirk, W. H. 1954. Index of treatment. 3rd ed. Bailliére, Tindall and Cox London, England.

MAYER, K. 1953. *In* Canine Medicine. American Veterinary Publications, Inc. Evanston, Illinois.

MELLANBY, E. 1947. Vitamin A and bone growth. J. Physiol. **105:** 382–399.

MORGAN, A. F. 1940. Deficiencies and fallacies in canine diet. North Am. Veterinarian. **21:** 476–483.

NORMAN, G. F. & A. MITTLER. 1948. Interrelationship of vitamin D and the sex hormones in calcium and phosphorus metabolism of rats. Proc. Soc. Exptl. Biol. Med. **67:** 104–111.

REINACH, N. & T. F. ADELAAR. 1955. Personal communication.

SCHNELLE, G. B. 1950. Radiology in Small Animal Practice. 2nd ed. North Am. Veterinarian. Evanston, Ill.

SCHULZ, K. 1952. Unpublished pathological report.

SWAIN, L. A. & D. R. IDLER. 1955. Progress Report of the Pacific Coast Stations of the Fisheries Research Board of Canada. No. 103.

THEILER, A. 1934. The osteodystrophic diseases of domesticated animals. Vet. J. **90:** 141–206.

THEILER, A., P. J. DU TOIT & A. I. MALAN. 1937a. Studies in the mineral metabolism XXXVIII. The influence of variations in the dietary phosphorus and in the Ca:P ratio on the production of rickets in cattle. Onderstepoort J. Vet. Sci. Animal Ind. **8:** 375–414.

THEILER, A., P. J. DU TOIT & A. I. MALAN. 1937b. Studies in mineral metabolism XXVIII. Calcium and phosphorus in the nutrition of growing pigs. Onderstepoort J. Vet. Sci Animal Ind. **9:** 127–164.

THOMAS, A. D. 1928. Several cases of so-called "bent-leg" in sheep. S. Africa J. Sci. **25:** 278–281.

IDIOPATHIC FAMILIAL OSTEOPOROSIS IN DOGS: "OSTEOGENESIS IMPERFECTA"*

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Osteogenesis imperfecta is a disease that occurs in small animals, especially members of the cat family, as well as in human beings.[1] Chance development of this condition in 6 of 7 members of a litter of standard poodle puppies several years ago provided an opportunity for study. Since that time we have been able to follow 2 other litters: 1, also of poodles, in which 4 of 12 puppies had the disease; and a second, of Norwegian elkhounds, of which 3 of 6 puppies had the disease. The diagnosis was confirmed in each instance by X ray of the extremities, which showed the changes characteristic of the disease. Several of the clinically uninvolved siblings studied by X ray were normal, except for evidence of mild rickets in 1.

Each of the 13 involved dogs displayed a clinical picture manifested by numerous pathological fractures and by the development of deformities. The fractures, usually occurring with minimal trauma, healed at a rapid rate, even though the dog continued to walk. Only 2 fractures, which occurred after trauma, required splinting. None of the dogs developed pseudoarthroses. The deformities were characterized by a flattening of the front paws and metatarsals to create a "snow shoe" appearance, an outward bowing of the elbows, and severe knock knees. It should be emphasized that these deformities are seen in other bone diseases in dogs, such as rickets,[1] or osteoporosis associated with copper deficiency,[2] and are not specific for osteogenesis imperfecta.

Despite the disease, most of the dogs achieved a height within an inch or 2 of their normal siblings. Two of the poodles, however, one from each litter, were decidedly stunted, one at birth, and the other by the time he reached 3 months of age. Except for the 1 runt, all of the dogs appeared to be entirely healthy until 6 to 8 weeks of age, when the bony abnormalities became apparent.†

Physical examinations were negative except for the skeletal system. Although the most severely ill dogs were reluctant to stand on their hind legs, this appeared to be due to pain rather than weakness. The extremities displayed unusual hypermobility. There was no articular involvement. The sclerae did not appear blue and, so far as we could tell, the dogs were not deaf.

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† By the fourth or fifth month of age, several of the puppies began to display other manifestations, which may or may not be related to the bone disease. Four puppies, 2 from each of the poodle litters, experienced cyclic regurgitation consisting of vomiting and then reconsuming their food as many as 20 times daily. In 1 of these dogs, gastrointestinal fluoroscopy and X ray, performed by G. Schnelle, disclosed massive dilation of the esophagus. Three puppies from the first litter developed diarrhea during their fourth or fifth month of age, for which no cause could be found. It should be emphasized that the bone disease was well established by the time these intestinal manifestations appeared. Whether or not these systemic manifestations are related to the bone disease is a matter of conjecture.

The family histories were negative except for the (cream-colored) stud of the first litter, who is said to have been weak in his hind legs. The bitch of the first litter had been bred on 3 former occasions to different studs, all black, and had had 28 normal puppies. The pedigrees of the 2 poodle bitches showed no interrelationship. The studs, however, were half brothers. There was no correlation of the severity of the disease with the sex of the puppies or with the color.

The diet of both poodle litters was extremely full, consisting of ground meat, milk, eggs, dog biscuit, and approximately 5 drops daily of a vitamin concentrate containing 1,700 units of vitamin D per cubic centimeter. Two of the involved siblings of the second litter were entirely bottle fed.

The clinical course of the dogs was of great interest. Every dog that attained 6 months of age began to improve. The fractures no longer occurred. The deformities became less apparent, especially the "snow-shoe" deformity and the knock knees, which almost entirely disappeared.

Laboratory Data

Routine laboratory studies were performed on 3 of the dogs. Urinalysis was normal. There was no albumin or sugar. The sediment contained rare white blood cells but no red blood cells or crystals. Specific gravity ranged as high as 1.028. Stools were semiformed and occasionally liquid. There was no excess of undigested muscle fibers or fat. Repeated examinations in our laboratory, that of the Angell Memorial Animal Hospital, Boston, Mass., and the Department of Tropical Public Health, Harvard School of Public Health, also in Boston, were all negative for ova and parasites. Of the 3 dogs studied from the hematological point of view (TABLE 1), 2 were anemic, with hemoglobins of 8.4 and 9.0 gm. per cent respectively. Red cell indices indicated a hypochromic microcytic anemia in both. The third dog had a negligible

TABLE 1

HEMATOLOGICAL AND CHEMICAL DATA ON THE BLOOD OF 3 DOGS 4 TO 5 MONTHS OLD WITH OSTEOGENESIS IMPERFECTA, AS COMPARED WITH REPORTED VALUES FOR NORMAL DOGS*

	1	2	3	Normal
Hgb.	8.4	9	11.3	12–18
Hct.	28.5	29.5	34	40–55
R.B.C.	5.59	6.01	5.3	6.4–8
M.C.H.	15	15	21.3	19–33
M.C.V.	51	49.1	64	64–72
M.C.H.C.	29.5	30.5	33.2	30–32.6
Ca (mg. %)	9.6	11.2	11.6	9–11
P (mg. %)	6.5	7.2		2.2–8
Alk. p'tase (units)	6.7	5.2		<8*
N.P.N. (mg. %)	18	15		17–38
T.P. (gm.%)	5.1	4.7	4.9	6.7
P.T. (sec.)	7		6	9–12
Serum Cu γ/100 ml.	20	37		38 to 58 (observed) 78 to 100 (reported)[2]

* The serum alkaline phosphatase level (Bodansky units) was found to be elevated in normal growing puppies.

anemia. Both anemic dogs displayed hematopoietic responses to ferrous sulfate, the former by a rise in circulating reticulocytes from 2 per cent to 3.5 per cent and elevation in hemoglobin to 9.3 gm. per cent in 1 week, and the latter by an elevation in hemoglobin to 10.8 gm. per cent after 2 months of therapy. Hematological studies on several control dogs maintained on average diets revealed anemias of equal or greater degree.

Chemical examination of the serum and blood performed in several dogs at 4 months of age showed normal calcium, phosphorus, and alkaline phosphatase concentrations. Total serum protein concentration was slightly decreased.

Serum copper determinations were performed in 2 of the dogs because of the similarity of the clinical and roentgenologic picture to that described by Baxter *et al.* in dogs suffering from severe dietary copper deficiency.[2] The determination in dog no. 1 was kindly performed for us by Christopher Earl, of the Department of Neurology, Boston City Hospital. The value recorded for dog no. 2 was an average of 4 determinations performed in our laboratory by the method of Gubler *et al.*,[4] the actual values ranging between 32 γ and 43 γ per 100 mg. Although these values are distinctly lower than the reported normal, they are only slightly lower than a series of 4 determinations performed on control dogs of comparable ages, selected at random. In order to exclude further the possibility that the dogs might be suffering from dietary copper insufficiency, or inability to absorb copper, the concentration of copper in the

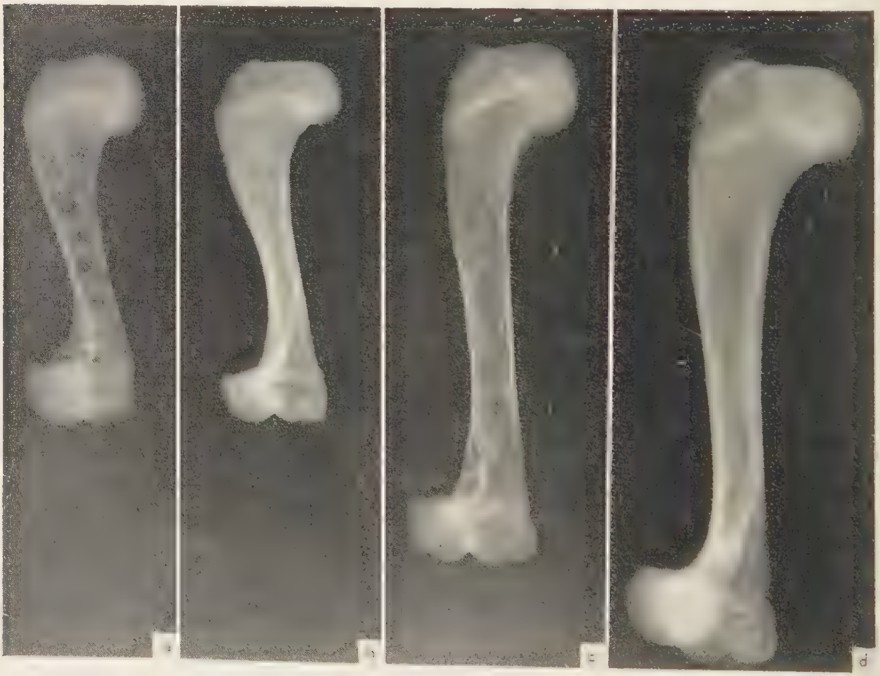

FIGURE 1. X rays of the humerus of (a) a 6-week-old puppy with osteogenesis imperfecta; (b) a normal cocker spaniel puppy approximately 3 months old, (c) a 5-month-old puppy with osteogenesis imperfecta; and (d) a 5-month-old mongrel. The films of the 4 bones were obtained simultaneously, with identical exposure. Chemical data on the bones are recorded in TABLE 2.

liver of dog no. 1 was determined by Earl. It was found to be normal, 46.9 γ per gram wet weight. This is to be compared with Baxter's observation that the concentration of copper in the liver of normal dogs is 24 to 48 γ per gram while, in the dogs fed a copper deficient diet, very low levels (0.9 to 3 γ per gram) were observed.[2]

There remains the possibility that the slightly low serum copper concentration in these dogs might reflect a deficiency of plasma caeruloplasmin, rather than dietary copper. For this reason the plasma caeruloplasmin of 1 of the dogs of the second litter, age 2 months, was compared with that of a normal puppy by Malcolm Thompson of our laboratory.[3] The values were in close agreement, 14 and 15 mg. per 100 ml., respectively.

Roentgenologic Picture

X rays of the bones showed a striking picture, as indicated in FIGURE 1. The extreme thinness of the cortex, most conspicuous in the youngest puppies, was still evident in the older dogs, especially in the region of the metaphases. Finally, however, by the age of 8 months the cortex was seen to become thicker and much more dense (FIGURE 2). Another abnormality was an apparent decrease in the number of delicate intramedullary trabeculae, giving the bones

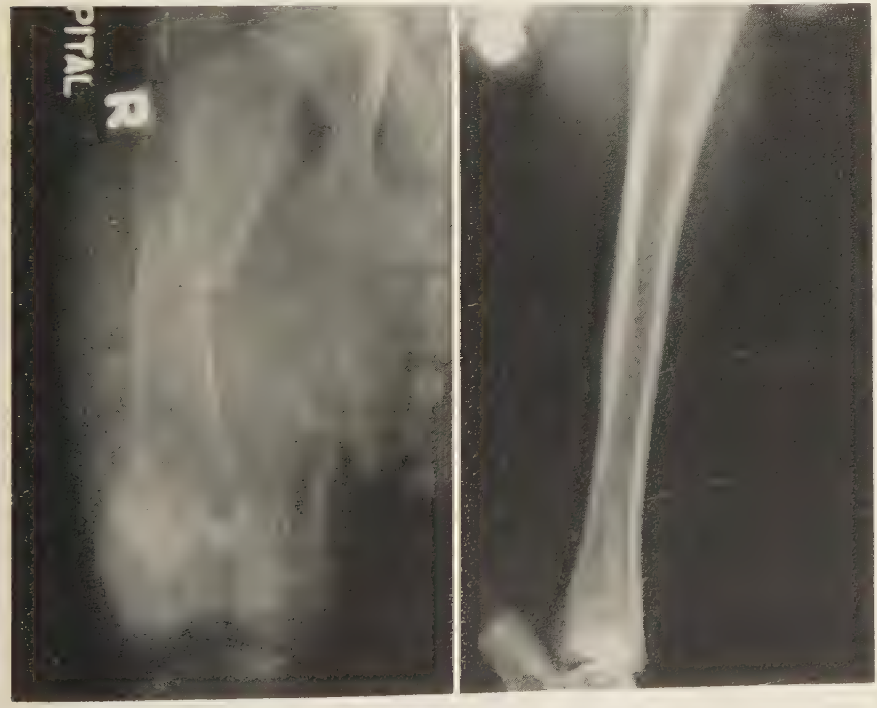

FIGURE 2. At the left is shown the X ray of the femur of a 6-week-old puppy following accidental fracture, prior to splinting. Note the exceedingly thin cortex and the severe osteoporosis. On the right is an X ray of the same bone when the dog had reached the age of 8 months. The cortex has become very dense and approximately normal in thickness. It appears to be somewhat thicker than normal at the site of the healed fracture.

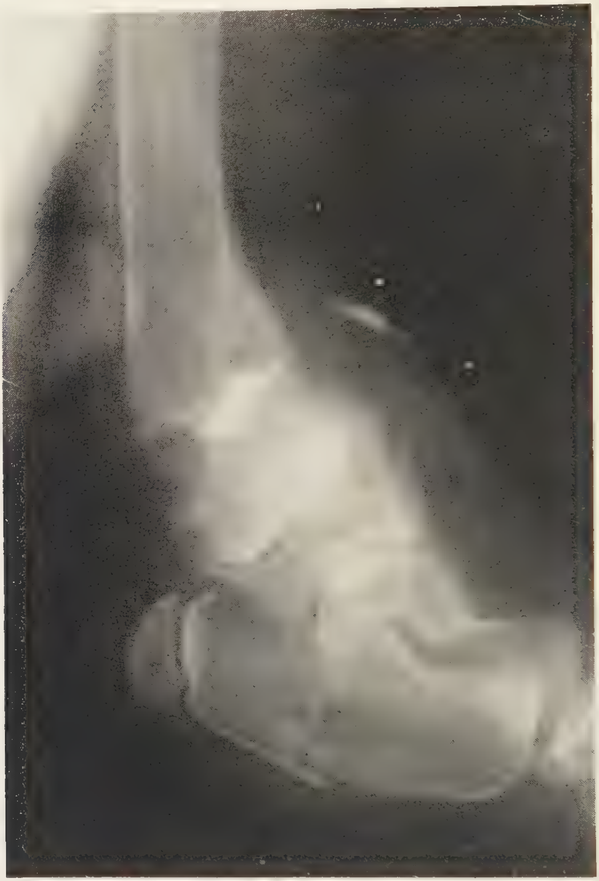

FIGURE 3. An X ray of the region of the olecranon in a 4-month old puppy with osteogenesis imperfecta who had not experienced recent injury, showing typical metaphyseal fracture and epiphyseal displacement at the lower end of the humerus, and a pathological fracture of the olecranon. The fractures occur at sites that are under stress during weight bearing. The increased density of the epiphysis of the humerus reflects coincidental mild-healing rickets.

a ground-glass appearance, marred by transverse lines of greater density, which may be the sequellae of previous fractures. In the metaphyseal area of most of the long bones there were sharply defined irregular marginal radiolucent zones, presumably fractures, and in many instances the epiphyses were partially dislocated (FIGURE 3). The fractures appeared to heal very rapidly, with relatively little callus formation. They were often followed by a residual bowing deformity.

Films of the clinically uninvolved sibling of the first litter showed the cortices to be of normal thickness. There were no diaphyseal fractures. There was definite increased density and some outward lipping of the epiphyses. This finding, also present in some of the involved dogs, is characteristic of healing rickets in the dog.[1]

These films were reviewed with Gerry Schnelle, who stated that they were entirely characteristic of osteogenesis imperfecta as he has seen it in animals.

Chemical Studies on Bones

Chemical studies of the bones were carried out in the following manner. The bones were cleaned carefully down to the periosteum. After weighing, they were immersed in water by means of a counterweight tared under water, and reweighed. The bones were then dried at 105° C. to constant weight, and ashed overnight at 500° C. After weighing, the ash was dissolved in 100 ml. concentrated HCl, filtered, diluted to 200 ml. with 10 per cent HCl, and analyzed for calcium and phosphorus by appropriate methods.[6, 7] From the weight of the bone in air and submerged it was possible to calculate the volume of the bone by the formula:

$$V = C + B - X$$

where V = volume of bone in cubic centimeters, C = weight (in grams) of the submerged counterweight, B = weight of the bone (in air), and X = the weight of the submerged bone and the attached counterweight. The specific gravity of the bone was then calculated, and the electrolyte content expressed as milligrams of calcium per cubic milliliters of bone.

The results of analysis of bones from 2 puppies with osteogenesis imperfecta, aged 6 weeks and 5 months, respectively, and of a number of normal dogs of various breeds, aged 2 months to 5 months, are shown in TABLE 2. To our surprise, the specific gravity of the 3 bones from the dogs with osteogenesis imperfecta, analyzed in this manner, was less than unity. This was confirmed by the observation that the humerus and femur of the diseased dogs floated in water, while normal bones sink. The most striking abnormality of the diseased bones was their calcium content per cubic milliliter of gross bone. It is of interest that both this value and all the values studied, except the specific gravity, were more abnormal in the 6-week-old puppy than in the one aged 5 months. The calcium-phosphorus ratio was essentially constant throughout, and approximated 1.9.

TABLE 2

CHEMICAL DETERMINATIONS ON BONES

	Osteogenesis imperfecta			Normal		
	Dog	Humerus	Femur	Dog	Humerus	Femur
Specific gravity	(A)	0.89		(E)	1.15	1.16
				(B)	1.00	1.01
	(C)	0.84	0.92	(D)	1.11	1.13
Calcium content (mg. per cc. of gross bone.)	(A)	19.4		(E)	53.8	56.0
				(B)	57.0	52.6
	(C)	41.5		(D)	51.5	50.2
Dry weight volume (mg. per cc. of gross bone)	(A)	0.25		(E)	0.44	0.44
				(B)	0.62	0.57
	(C)	0.43	0.37	(D)	0.63	0.64
$\frac{\text{Ash weight}}{\text{Dry weight}} = \%$	(A)	30.2		(E)	42.7	44.0
				(B)	46.5	44.0
	(C)	33.1	31.0	(D)	46.6	48.3

The ratios indicated at the left were calculated for the humerus and femur of 3 normal puppies: (E), 6 weeks old; (B), 3 months old; and (D), 5 months old, and of 2 dogs with osteogenesis imperfecta: (A), 6 weeks old; and (C), 5 months old. The lettering corresponds with that used in FIGURE 1.

Pathology

The pathological changes in the diseased poodles 3, 4, and 5 months of age were compared with the findings in a group of normal dogs of various breeds, aged 2 weeks, 1 month, 2 months, $3\frac{1}{2}$ months, and 5 months, respectively. Viewed under low power, the involved bones demonstrated the abnormalities one would expect from the X rays: exceedingly thin cortices; thin and sparse cancellous bone, especially in the area of the metaphyses; and fractures occurring through almost every metaphysis (FIGURE 4). Some of these fractures involved only the cortex, while others were extensive enough to disrupt the whole metaphysis, with, in 1 case, aseptic necrosis of the whole metaphyseal region. It should be pointed out that several of the control dogs also showed evidences of fractures in the metaphyses of the long bones. These were much smaller and less frequent than in the dogs with osteogenesis imperfecta, and may have been due to minimal rickets.

Comparison of sections of bones of the diseased dogs with the analogous bones of normal dogs of various ages showed a striking fact. Although the bones of the diseased and normal dogs were roughly of the same size, the stage of development of bone in the involved dogs appeared to be from 1 to 2 months behind that of the normal. This is shown by comparing cross sections of the shaft of the humerus. At 3 months of age, when the normal humerus had a thick cortex of cancellous bone with a well-formed medullary cavity, the sick dog had a very poorly organized, thin cortex of loose cancellous bone, with persistence of trabeculae in the medulla (FIGURE 5). At 4 months, when the normal dog had a very thick cortex that was beginning to develop Haversian systems, the sick dog had developed a good medullary cavity but its cortex was still predominately trabecular bone. At 5 months the control dog had developed a very dense, well-organized cortex. The sick dog, at 5 months of age, approached normal, but the cortex was much more loosely packed and still contained some trabecular bone. Longitudinal sections of the cortex emphasize this difference.

Higher magnification of the epiphysis showed two important deviations from normal. The first was definite narrowing of the epiphyseal plate, apparently due to a diminution in the numbers of both the proliferating and degenerating cartilage cells. This abnormality was especially striking in the 3-month-old dog, which was moderately stunted in growth. Sections in nonstunted dogs 4 and 5 months of age showed epiphyseal plates of more nearly normal width. The individual steps in the processes of maturation of the cartilage appeared qualitatively normal. The cells were arranged in orderly columns, and the invasion by the capillaries, with the opening of the chondrite capsules, was regular and normal (FIGURE 6). The columns of calcified cartilage were regular in their distribution, and this calcified lattice persisted well into the metaphysis. Periodic acid-Schiff and metachromatic stains of this area showed the cartilage to exhibit its normal staining reactions. Alkaline-phosphatase stains of this area, performed by Richard Cohen, were also normal, as compared with suitable control.

The second and more constant abnormality was a decrease in the number of

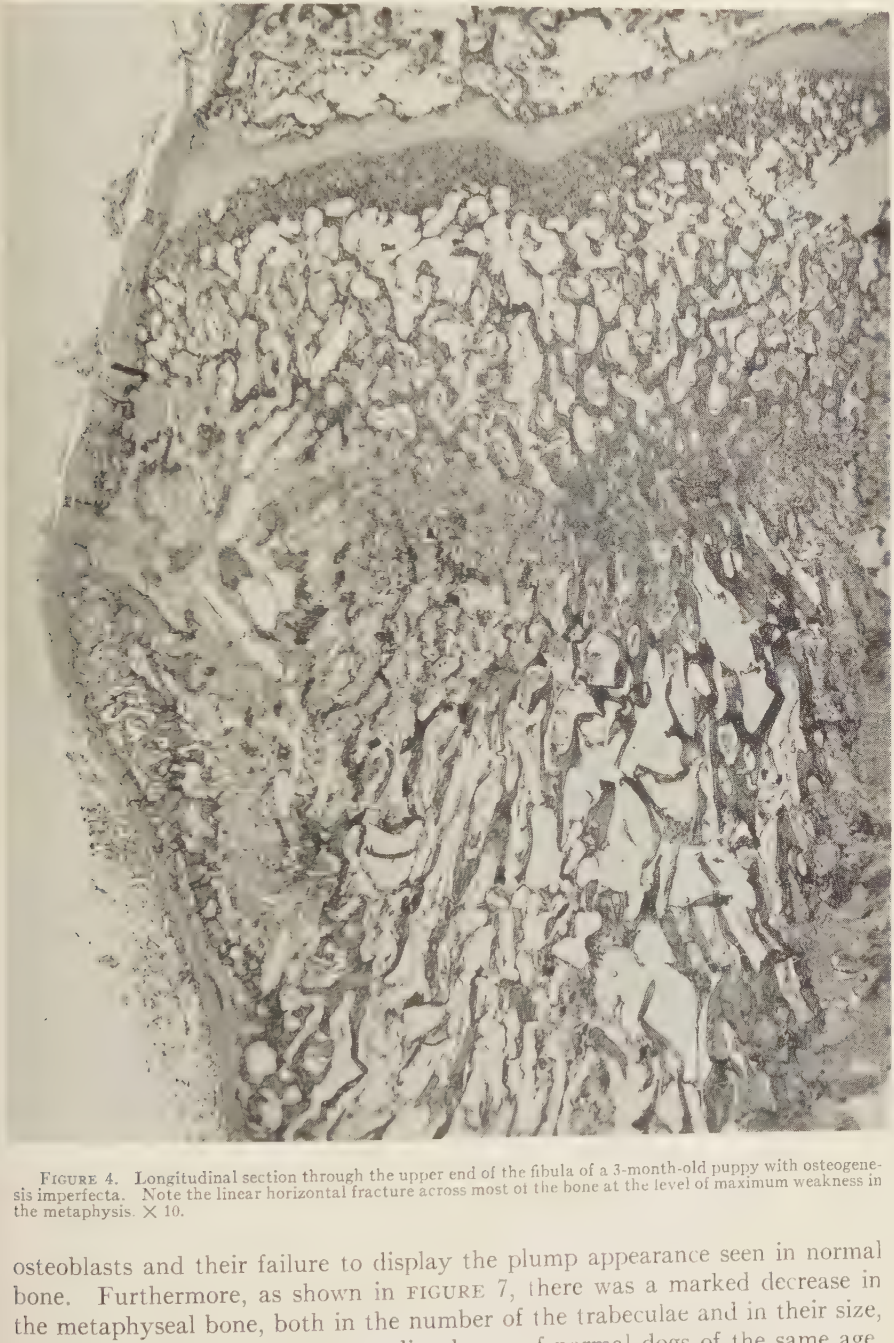

FIGURE 4. Longitudinal section through the upper end of the fibula of a 3-month-old puppy with osteogenesis imperfecta. Note the linear horizontal fracture across most of the bone at the level of maximum weakness in the metaphysis. × 10.

osteoblasts and their failure to display the plump appearance seen in normal bone. Furthermore, as shown in FIGURE 7, there was a marked decrease in the metaphyseal bone, both in the number of the trabeculae and in their size, in comparison with the corresponding bones of normal dogs of the same age.

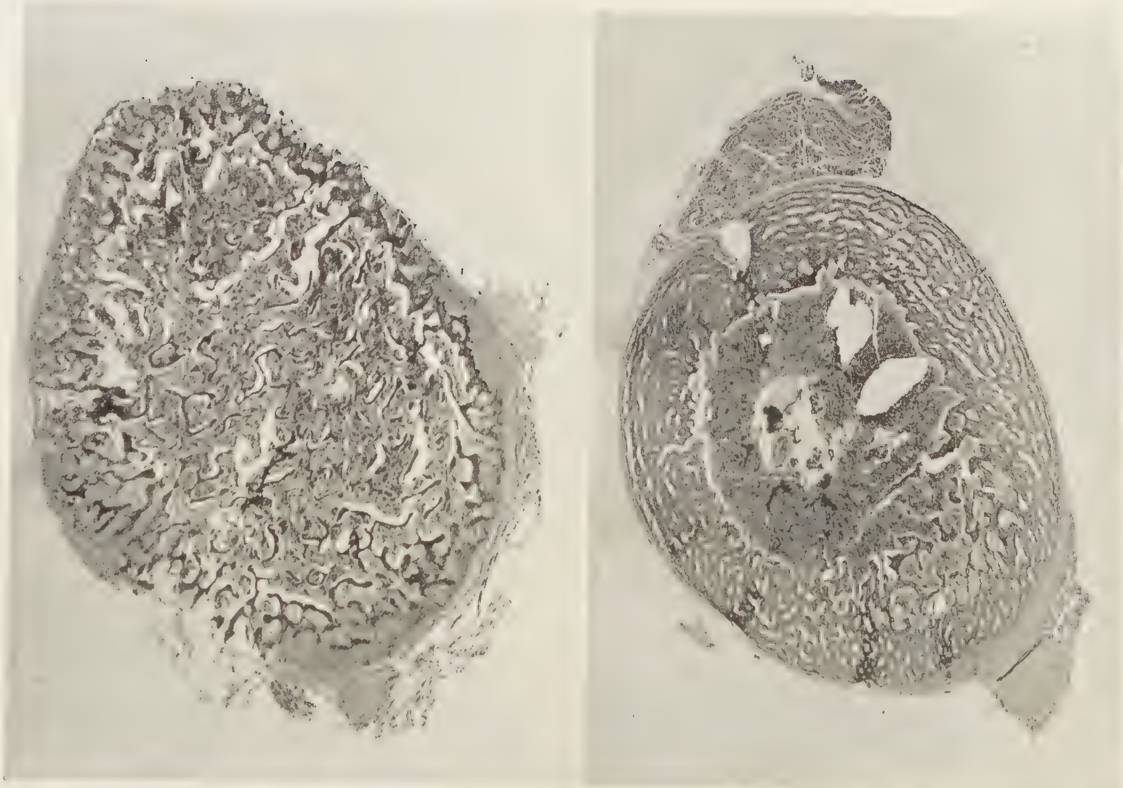

FIGURE 5. On the left is a cross section of the humerus of a 3-month-old dog with osteogenesis imperfecta. On the right is a corresponding section from a normal dog of the same age. Note that the normal dog has formed a well marked cortical layer, with beginning formation of compact bone and a medullary canal. The dog with osteogenesis imperfecta displays almost complete absence of cortical development × 8.

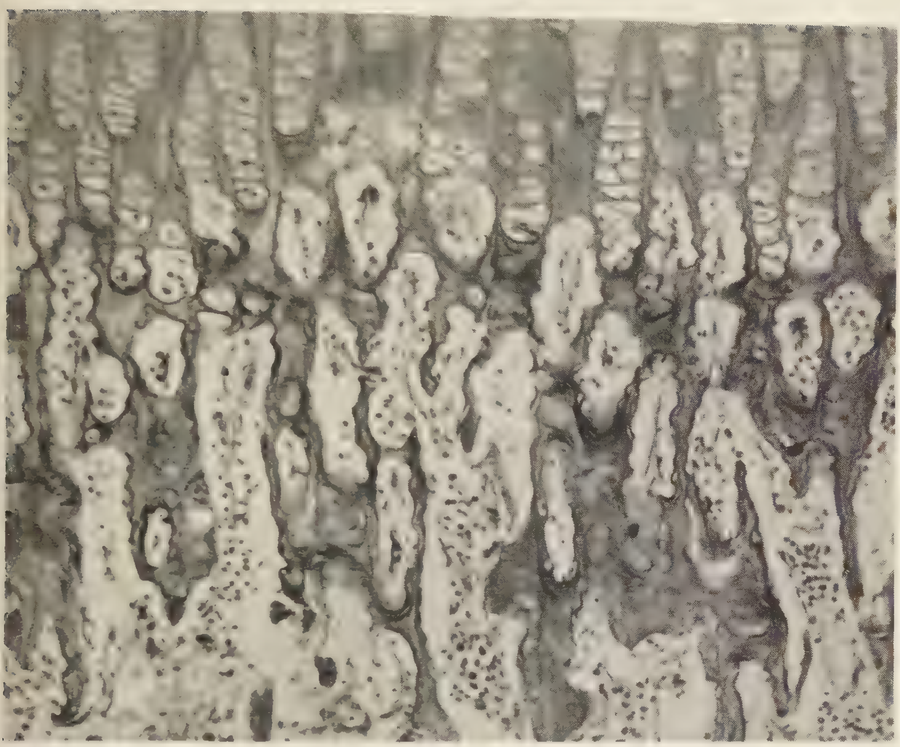

FIGURE 6. High magnification of the epiphyseal plate of the 3-month-old puppy with osteogenesis imperfecta, showing normal maturation of the cartilagenous cells and invasion by capillary tufts. × 120.

Study of the metaphyses by means of the polarizing microscope showed that there was much less birefringent material in the trabeculae of the sick dog than in the normal dog of the same age. The amount of birefringent material in the metaphysis of the rib of the sick dog, aged 3 months, more nearly approximated that seen in a corresponding area of the rib of the 2-week-old control dog. Use of the first-order red plate showed that the birefringent areas were positive with respect to the long axis of the trabeculae.

The development of membranous bone (in the skull and jaw) appeared to be qualitatively normal, but here again the production of bone was quantitatively depressed. The dogs continued to develop and lose teeth at the normal rate and time, and the dentine, cementum, peridontal membrane, and odontoblastic layer of the permanent teeth were normal. The enamel organ was normal except where involved in a fracture. The presence of small cartilagenous plates in relation to the developing teeth would seem to indicate that, up to the stage of osteogenesis, the development of the jaw was normal. On the other hand the organization of the alveolar bone and cortical bone of the involved dogs resembled that of normal dogs at a much earlier age. There were numerous fractures in the mandibles, both the horizontal and vertical rami.

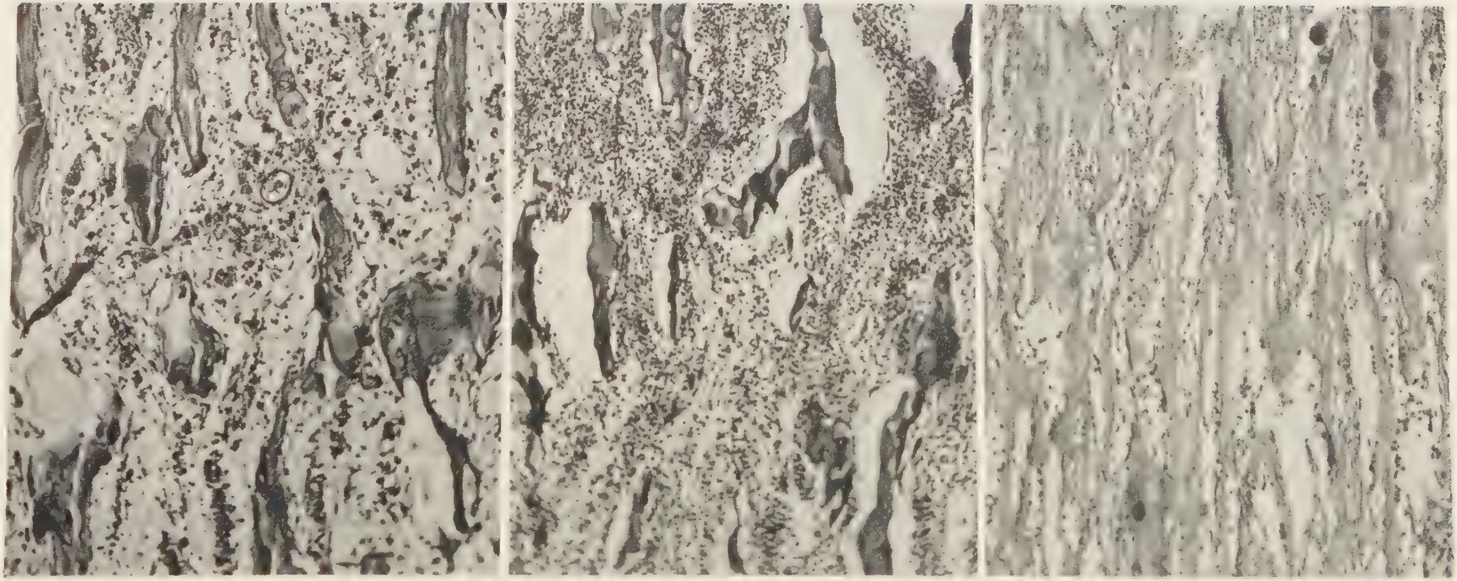

FIGURE 7. Corresponding areas of the metaphysis of the rib of a normal puppy 2 weeks of age (left), a 3½-month-old dog with osteogenesis imperfecta (center), and a 4-month-old normal dog (right) are compared, using equal magnification. H & E stain. Note the sparse, thin trabeculae in the involved dog, producing a picture very similar to that of a normal dog 3 months younger. × 50.

Complete autopsies were performed on 2 of the involved dogs. Gross and histological study of the soft tissues, by ordinary techniques, failed to disclose any striking abnormalities. Comparative studies with tissues of normal dogs of comparable ages were not undertaken.

Discussion

Although one cannot state that the disease we have described in dogs is identical with osteogenesis imperfecta, as seen in humans, there are a surprising number of similarities between the 2 conditions. The marked thinness of the cortices, with numerous pathological fractures, during puppyhood or childhood; the rapid healing, usually with lesser amounts of callus; the gradual development of deformities, chiefly bowing of the bones; the striking flaccidity of the ligaments and joints; and the spontaneous improvement at puberty are all characteristics common to both diseases. There are, of course, certain differences, notably the high incidence of epiphyseal displacement and distortion in the involved dogs, an unusual finding in humans with osteogenesis imperfecta, and the absence of widening of the metaphyseal shaft, often seen in the human disease. Whether these are merely species differences or represent a fundamental difference in the nature of the disease is unknown. It should be kept in mind, in this connection, that dog bones exhibit considerably more longitudinal growth than do human bones, and also that dogs commence weight bearing at a time when the metaphyseal cortex is exceedingly thin and most of the support of the metaphysis is derived from the calcified cartilage and the young bony trabeculae.

Pathologic study of the dogs was facilitated by the fact that the bones of the involved animals, 3, 4, and 5 months of age, could be compared with normal controls ranging from 2 weeks to 5 months of age. The chief abnormality appeared to be inhibition or delay of osteogenesis, manifested by a reduced number and apparent activity of the osteoblasts and by a sparseness and thinness of the trabeculae. The observation of the markedly reduced birefringence of the metaphyseal trabeculae of the involved dogs suggests the possibility that there is less collagen being laid down in the presence of the disease, or that it is being laid down at a slower rate. This deduction, if correct, would be consistent with Follis' observation that in osteogenesis imperfecta in humans "The defect is related to the formation of collagen."[8]

Either because of, or in addition to this defect, the involved dogs displayed a retardation in the development of bone, so that it presented a histologic picture resembling in many respects that of normal bone of dogs 1 or 2 months younger. The concept that osteogenesis imperfecta represents a delay in the developmental formation of bone has also been suggested with regard to the disease in humans.[9]

Although there was definite narrowing of the epiphyseal plate especially in the most stunted dog, the fact that all but 1 of the 12 other dogs studied attained near-normal height indicates that there was no serious defect in the development of the cartilagenous model. The normal development of the cartilagenous plates in the jaw and the orderly appearance of the cartilage cells and of the calcified cartilage in the epiphyseal plate are further evidenc that cartilage was not seriously involved.

Chemical studies of the bones disclosed a striking deficiency both in tota protoplasm (as estimated from the dry weight) and in calcium and phosphorus This was less conspicuous in the bones of the older of the involved dogs.

The studies presented above demonstrate that the clinical, roentgenologi chemical, and pathologic manifestations of this disease become much less ap parent after the animal reaches puberty, which is at the age of 6 or 7 month in dogs. If one accepts the fact that the disease is manifested primarily by delay in the process of osteogenesis, it is not surprising that, with time, as th epiphyses close and the wave of osteogenesis finally catches up to the carti lagenous model, the deficits of the disease become much less marked.

The roentgenologic picture of the dogs with osteogenesis imperfecta closel resembles that described in dogs with copper deficiency.[2] Pathological studie of the epiphyses of bones of dogs with the latter condition[10] revealed a con sistent increase in the width of the zones of both proliferating and matur cartilage. On the other hand, the most striking change in copper deficienc as in osteogenesis imperfecta, was thinness of the cortices and of the trabecula especially of the metaphysis. Although the age of the copper-deficient dog at the time of sacrifice is not mentioned in the report,[10] one gathers from th previous experiments that they must have been approximately 5 months ol By this time even the dogs with osteogenesis imperfecta have developed fairl mature cortices in their long bones. It is therefore difficult, from the data a hand, to relate the developmental changes due to copper deficiency to thos seen in osteogenesis imperfecta. All that can be said is that the former cond tion, like the latter, appears to be associated with some interference with th process of osteogenesis.[11]

Whether the osseous changes in our dogs were related in any way to coppe metabolism is difficult to determine with certainty. In view of the full die employed and from the fact that the liver-copper level was normal in the do with osteogenesis imperfecta, it would seem unlikely that the dogs could b suffering from dietary copper deficiency. The anemia exhibited by some of th dogs with osteogenesis imperfecta is almost certainly due to coincident iro deficiency, and is not related to the anemia reported in copper-deficient dogs. The significance of the slightly reduced serum-copper level in the dogs wit osteogenesis imperfecta is therefore uncertain. While it is possible that som alteration in copper metabolism is the site of the defect in osteogenesis im perfecta, it seems much more likely that both conditions, although not directl related, affect the same common but as yet poorly defined site.

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References

1. Schnelle, G. B. 1950. Radiology in Small Animal Practice. A text on applied radiography and diagnosis. 2nd ed. North Am. Veterinarian. Evanston, Ill.
2. Baxter, J. H. & J. H. Van Wyk. 1953. A bone disorder associated with copper deficiency. I. Gross morphological, roentgenological, and chemical observations. Bull. Johns Hopkins Hospital. **93:** 1–23.
3. Dukes, H. H. 1947. The Physiology of Domestic Animals. 6th ed. Comstock. Ithaca, N. Y.
4. Gubler, C. J., M. E. Lahey, H. Ashenbrucher, G. E. Cartwright & M. M. Wintrobe. 1952. J. Biol. Chem. **196:** 209–220.
5. Scheinberg, I. H. & M. T. Thompson. To be published.
6. Fiske, C. H. & Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. **66:** 375–401.
7. Fiske, C. H. & M. A. Logan. 1931. The determination of calcium by alkalimetric titration. II. The precipitation of calcium in the presence of magnesium, phosphate, and sulfate, with applications to the analysis of urine. J. Biol. Chem. **93:** 211.
8. Follis, R. H., Jr. 1952. Osteogenesis imperfecta congenita. A connective tissue diathesis. J. Pediat. **41:** 713–721.
9. Weber, M. 1930. Osteogenesis imperfecta congenita. A study of its histopathogenesis. Arch. Pathol. **9:** 984–1006.
10. Baxter, J. H., J. J. Van Wyk & R. H. Follis, Jr. 1953. A bone disorder associated with copper deficiency. II. Histological and chemical studies on the bones. Bull. Johns Hopkins Hospital. **93:** 25–39.
11. Follis, R. H., Jr. 1953. Histochemical studies on cartilage and bone. III. Bull. Johns Hopkins Hospital. **93:** 386–391.
12. Van Wyk, J. J., J. H. Baxter, J. H. Akeroyd & A. G. Motulsky. 1953. The anemia of copper deficiency in dogs compared with that produced by iron deficiency. Bull. Johns Hopkins Hospital. **93:** 41–49.

INFLUENCE OF VITAMIN D ON SERUM CALCIUM AND INORGANIC PHOSPHATE IN THE NEONATAL PERIOD*

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In 1923 Park and his associates[1] studied the effect of cod liver oil administered to young rats receiving diets of varying composition of calcium and phosphorus. They found that cod liver oil, when given to rats whose diets had either a low content of calcium or of phosphorus or of both elements enabled the animals to maintain normal serum calcium and phosphorus levels. They concluded that the calcifying factor in cod liver oil exerted a homeostatic control on the utilization of calcium and phosphorus. This control was so efficient that even with minimal amounts of Ca and P in their diets, the animals maintained normal serum Ca and P levels.

Kramer and Howland[2] in 1925 repeated and extended these experiments. The results of these studies were reported by Kramer in 1932. Large series of animals were given diets varying in Ca-P ratio and varying in the amounts of butterfat and cod liver oil. They found that in the absence of vitamin D in the diet, the serum Ca and P is practically under the control of the dietary content of these elements, that is, a diet low in Ca or P will produce a low serum Ca or low-serum P. The administration of vitamin D enabled the animals to raise the serum Ca or P levels or of both in spite of the discrepant content of these elements in the diet.

Sobel and Hanok,[3] studying the relation of diet to the composition of blood and teeth in young rats, also found that on diets high in Ca and low in P, or low in Ca and high in P, or whether low in Ca and P, or whether the animals were on these diets for 30 or 45 days, vitamin D raised the serum levels of Ca and P. Without vitamin D in the diet, the serum Ca and P levels were determined by the concentrations of these elements in the diet.

Investigators[4-9] in the field of rickets showed that vitamin D, whether given in the form of cod-liver oil, irradiated ergosterol, irradiated food, or in the form of irradiation of the skin, prevented or cured the disease no matter what the concentration of Ca or P or the Ca-P ratio in the diet might be.

Following the discovery of the fact that vitamin D will cure rickets, many diseases that resembled rickets serochemically, pathologically, and radiographically were thus treated. Renal diseases, metabolic disorders as seen in the Fanconi syndrome, disorders of bone associated with lack of phosphatase activity in the serum, and diseases of the parathyroids (hypoparathyroidism) were treated with vitamin D. Vitamin D in the usual therapeutic amounts, when administered to patients suffering from the above disturbances, failed to affect the serum Ca and P levels or to influence the rachitic process in the bones. The prophylactic administration of vitamin D also failed to prevent rickets from developing in infants born in families having resistant rickets. Vitamin D in extremely high concentration (100,000 to 600,000 U.S.P. units), however, did influence favorably the rachitic process associated with the above-mentioned conditions.

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The mechanism of the action of vitamin D was studied by many investigators. Harrison and Harrison[10] showed that vitamin D acts on the renal tubules and enables the animals to resorb the phosphate filtered by the glomeruli. It has also been shown that in the absence of parathyroids there is maximum absorption of phosphate by the renal tubules.[11] The administration of parathyroid extract produces a phosphorus diuresis. Thus vitamin D appears to have an antagonistic effect towards parathormone.[12] It was also found, however, that vitamin D in excessively large amounts, when administered to animals or humans, produces parathormonelike results, that is, a loss of phosphorus in the urine. Vitamin D therefore has a dual effect depending upon the amount administered.

In 1936 an infant having neonatal tetany was studied by us.[13] The child was on breast milk for the first 5 weeks of its life and then was weaned and put on a whole-milk mixture. Antirachitic supplement had been given since the fifth week of life. The infant developed convulsions during his sixth week of life and was admitted to the hospital. The serum Ca was 6.2 and serum P was 8 mg. 100 cc. of serum. X rays of the long bones were normal. The infant was studied metabolically. When the intake and output of Ca and P were measured in the 2 preliminary periods consisting of 3 days each, it was found that the partition of Ca and P in the urine and feces resembled that observed by Aub *et al.*[14] in cases of hypoparathyroidism. When parathyroid extract was administered, a marked phosphorus diuresis occurred and the serum Ca and P became normal. When parathyroid extract was withheld, again the convulsions recurred, and the serum Ca and P returned to the previous abnormal levels. The partition of Ca and P returned to what they were in the preliminary period. We tentatively concluded that tetany of the newborn resembled a state of hypoparathyroidism. A year later, Bakwin[15] found that when he administered inorganic P to newborn infants in the first week of life, the P level of the serum was raised to such a degree that it reciprocally affected the calcium. He also concluded that the infants during the neonatal period seemed to behave as do patients suffering from hypoparathyroidism. Barnett *et al.* and others[16-19] studied kidney function of the newborn infant. They found that the infant kidney glomerular filtration rate is about 33 to 40 per cent of that found in the normal adult.

Gardner *et al.*[20] and Gittleman *et al.*[21] showed that breast milk that contains a Ca P ratio of 2:1 and a low Ca and P concentration protects newborn infants against the occurrence of neonatal tetany.

In the course of studies on the effect of various diets on the serum Ca and P levels of infants during the first week of life, we found that when fluid cow's milk not containing vitamin D was given to infants, a lower incidence of hypocalcemia was observed than in infants fed a fluid cow's milk enriched with vitamin D (TABLE 1). This finding led us to a study of the effect of vitamin D

TABLE 1

INCIDENCE OF HYPOCALCEMIA IN INFANTS FED FORMULAE CONTAINING AND NOT CONTAINING VITAMIN D DURING THE FIRST WEEK OF LIFE

Milk	No. of infants studied	Number of hypocalcemics*	Per cent
Evap. milk enriched with vitamin D	41	10	24.4
Powdered milk enriched with vitamin D	60	10	16.6
Powdered milk, no vitamin D	60	5	8.3
Fluid cow's milk enriched with vitamin D	30	4	13.3
Fluid cow's milk, no vitamin D	75	4	5.3

* Eight mg. per 100 ml. of serum or less.

TABLE 2

INCIDENCE OF HYPOCALCEMIA IN INFANTS FED POWDERED MILK FORMULAE CONTAINING AND NOT CONTAINING VITAMIN D DURING THE FIRST WEEK OF LIFE

Milk	No. of infants studied	Number of hypocalcemics*	Per cent
Powdered milk, no vitamin D	60	5	8.3
Powdered milk enriched with vitamin D (400 U. S. P. units per quart)	60	10	16.6
Powdered milk + vitamin D (600 U. S. P. units per day)	60	14	23.3

* Eight mg. per 100 ml. of serum or less.

TABLE 3

INCIDENCE OF HYPOCALCEMIA IN BREAST-FED INFANTS RECEIVING AND NOT RECEIVING SUPPLEMENTS OF 600 U. S. P. UNITS OF VITAMIN D DAILY DURING THE FIRST WEEK OF LIFE

Milk	No. of infants studied	Number of hypocalcemics*	Per cent
Breast milk, no vitamin D	27	0	0
Breast milk + vitamin D (600 U.S.P. units)	15	0	0

* Eight mg. per 100 ml. of serum or less.

on the incidence of hypocalcemia and hyperphosphatemia in the first week of life.[22]

Infants in the first week of life were placed on a processed milk formula containing no vitamin D. These infants were compared with others receiving the same diet enriched with 400 units of vitamin D per quart of diluted formula, and also with a group of infants receiving the powdered-milk mixture supplemented with 600 units of vitamin D daily (TABLE 2). The incidence of hypocalcemia was significantly higher in the group receiving the supplement of vitamin D. Breast-fed infants were used as controls in these studies (TABLE 3). The group of breast-fed infants given a supplement of 600 units of vitamin D showed a tendency toward a higher level of P and a lower level of Ca, but not to the hypocalcemic levels.

It would therefore appear that vitamin D, when administered to infants during the first week of life, does not exert a homeostatic-regulating beneficial effect on the serum Ca and P. On the contrary, it worsens the tendency to hypocalcemia. Assuming that vitamin D acts on the renal tubules as an antagonist to parathormone, an attractive explanation for the paradoxical effect of vitamin D in the newborn suggests itself.

If a state of transient hypoparathyroidism of varying degree of severity exists during the first week of life, then the administration of vitamin D in the accepted therapeutic doses during this interval would tend to aggravate the hypoparathyroid state and would therefore cause the infants to have a greater tendency toward a lowering of the serum Ca and raising of the P levels.

References

1. Park, E. A., R. A. Guy & G. F. Powers. 1923. Proof of regulatory influence of cod liver oil on calcium and phosphorus metabolism. Am. J. Diseases Children. **26:** 101.
2. Kramer, B. & J. Howland. 1932. Factors which determine the concentration of calcium and of inorganic phosphorus in the blood serum of rats. J. Nutrition. **5:** 39.
3. Sobel, A. E. & A. Hanok. 1948. Calcification of teeth. 1. Composition in relation to blood and diet. J. Biol. Chem. **176:** 1103.
4. Guy, R. A. 1923. The history of cod liver oil as a remedy. Am. J. Diseases Children. **26:** 112.
5. Steenbock, H. & A. J. Black. 1924. Fat soluble vitamins. XVII. The induction of growth promoting and calcifying properties in a ration by exposure to ultraviolet light J. Biol. Chem. **61:** 405.
6. Steenbock, H. & M. T. Nelson. 1924. Fat soluble vitamins. XIX. The induction of calcifying properties in a rickets producing ration by radiant energy. J. Biol. Chem. **62:** 209.
7. Hess, A. F. 1924. Experiments on the action of light in relation to rickets. Am. J. Diseases Children. **28:** 517.
8. Kramer, B. 1925. Irradiated milk. Am. J. Diseases Children. **30:** 195.
9. Huldschinsky, K. 1920. Die Beeinflüssung der Tetanie durch Ultravioletlicht. Z. Kinderheilk. **26:** 207.
10. Harrison, H. E. & H. C. Harrison. 1941. Renal excretion of inorganic phosphate in relation to action of vitamin D and parathyroid hormone. J. Clin. Invest. **29:** 47.
11. Crawford, J. D., M. M. Osborn, N. B. Talbot, M. L. Terry & M. F. Morrill. 1950. The parathyroid glands and phosphorus homeostasis. J. Clin. Invest. **29:** 1448.
12. Crawford, J. D., D. Gribetz & N. B. Talbot. 1955. Mechanism of renal tubular phosphate reabsorption and the influence thereon of vitamin D in completely parathyroidectomized rats. Am. J. Physiol. **180:** 156.
13. Pincus, J. B. & I. F. Gittleman. 1936. Infantile tetany. A metabolic study. Am. J. Diseases Children. **51:** 816.
14. Aub, J. C., F. Albright, W. Bauer & E. Rossmeisl. 1932. Studies of calcium and phosphorus metabolism. VI. J. Clin. Invest. **11:** 211.
15. Bakwin, H. 1937. Tetany in newborn infants. Am. J. Diseases Children. **54:** 1121.
16. Barnett, H. L., H. K. McNamara & R. Hare. 1948. Measurement of glomerular filtration rate in premature infants. J. Clin. Invest. **27:** 691.
17. Dean, R. F. A. & R. A. McCance. 1947. Inulin, diodone, creatinine and urea clearances in newborn infants. J. Physiol. **106:** 431.
18. Rubin, M. I., E. Bruck & M. Rapoport. 1949. Maturation of renal function in childhood. J. Clin. Invest. **28:** 1144.
19. Barnett, H. L. 1950. Kidney function in young infants. Pediatrics. **5:** 171.
20. Gardner, L. I., E. A. MacLachlan, W. Pick, M. L. Terry & A. M Butler. 1950. Etiologic factors in tetany of newlyborn infants. Pediatrics. **5:** 228.
21. Gittleman, I. F. & J. B. Pincus. 1951. Influence of diet on the occurrence of hyperphosphatemia and hypocalcemia in the newborn infant. Pediatrics. **8:** 778.
22. Pincus, J. B., I. F. Gittleman, A. E. Sobel & E. Schmerzler. 1954 Effects of vitamin D on the serum calcium and phosphorus levels in infants during the first week of life. Pediatrics. **13:** 178.

OSTEOGENETIC PROPERTIES OF ESTROGENIC HORMONES*

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Specific effects of estrogenic hormones upon the formation of bone have been well described in birds, mice, and rats, and in each case the effects observed have been limited to the bone-marrow cavities, especially of the long bones. In birds, abundant proliferation of endosteal new bone is directly related to estrogen production during the egg-laying cycle. This phenomenon was first discovered in 1934 by Kyes and Potter,[1] who were studying ovulating pigeons. These investigators correlated the amount of medullary bone formation with the size of the ovarian follicles.

Subsequently, medullary bone formation in the pigeon and in other birds was studied in detail by Bloom, Bloom, and McLean,[2] and by others. It was found that in the pigeon formation of ovarian follicles is associated with a variety of other events in preparation for the laying of eggs. During the maturation of the ovarian follicles there occurs an ingrowth of bone into the marrow cavity from the endosteal surfaces of the long bones, coincident with a rise in the calcium content of the serum. Bone-free interstices remain in the marrow cavity for functioning bone marrow, although the structure continues throughout the marrow space.

This process for calcium storage continues until the eggshell is ready to be calcified. The serum calcium falls as the eggshells become calcified, and much of the endosteal bone disappears. The rise in the serum calcium during the egg-laying cycle is due to an increase in the nondiffusible, nonionized fraction. The calcium ion concentration of the plasma remains normal, as does the equilibrium between the ionized and the protein-bound calcium. The added calcium responsible for the hypercalcemia appears to be neither physiologically active nor in equilibrium with the other fractions. At the completion of the egg-laying cycle, any bone that remains in the bone-marrow cavity is destroyed, and the entire system reverts back to its resting state.

The situation is more complex in the laying hen. A system of medullary bone develops, but there is a continuous process of both storage and destruction since the hen needs the calcium for deposition of the eggshell at more frequent intervals and over a longer period of time. The phenomenon of medullary bone formation in the hen or in the pigeon, as well as in any other animals, has no relation whatsoever to any known effect of the growth hormone. It is therefore possible to eliminate from consideration any osteogenetic effect through the growth hormone.

Medullary bone formation also occurs in the mouse, following administration of estrogen.[3, 4] During the formation of endosteal bone, however, there is no elevation in the calcium content of the serum. There occurs an ingrowth of bone into the marrow cavity, as in birds, but the histologic picture is somewhat different. New bone formation arises from the endosteum lining the shafts of the long bones, and advances concentrically toward the axis of the shaft, following the vascular pattern of the bone marrow as it advances. The new bone encroaches upon the vascular areas of the marrow, completely disrupting its pattern and organization. Small areas of bone marrow, however, remain between the spicules of bone. As in the bird, new osteoblasts appear to develop by transformation of the reticular or primitive connective tissue cells of the bone-marrow stroma. The spongiosa is longer and denser than in the normal mouse, and in the early stages, the new endosteal bone consists of branches of new trabeculae between the old spicules. A certain amount of the new bone contains cores of unabsorbed cartilage matrix, indicating retardation of the normal process of bone resorption at the epiphyseal lines. With long-continued administration of estrogens, complete obliteration of the marrow cavities of the long bones can be produced. Changes in the alveolar bone are similar to those in the long bones.[5, 6]

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Recent studies with radioactive estrone[7] have been complicated by the fact that the distribution and localization of the steroids are more intimately related to steroid metabolism and to the excretion of the steroids and their metabolic products than to their specific affinity for the target organs of their physiologic activity. Six hours after a dose of 0.1 mg. (0.27 μc) of estrone-16-C^{14}, radioactivity is diffusely distributed throughout the soft tissues, including the ovaries and uterus, and it is still present in these tissues at 12 hours after injection. At 24 hours, however, no radioactivity can be detected in the ovaries or uterus, although uterine weights show evidence of the physiologic response to the steroid. Radioactivity has been found in the femur as early as 4 hours after administration of estrone, and significant amounts of activity persist in the liver, adrenals, long bones (including bone marrow), lymph nodes, and blood for 96 hours or longer.

There is no evidence that C^{14} is deposited in bone in the form of carbonate, nor that the radioactive carbon atom is excreted as $C^{14}O_2$. Since radioactivity is present in both uterus and bone as early as 4 to 6 hours, and disappears quickly from the uterus, the action on a target organ can be assumed to be initiated rapidly. The longer persistence in bone is compatible with a more prolonged action in this tissue. The finding of radioactive estrone and its metabolites in ether extracts of homogenized bone[8] strongly suggests an interaction between the estrone and some cellular fraction such as the osteogenetic connective tissue cells of the primitive bone marrow. During endosteal bone formation, the largest amount of radioactivity is found in association with the spongy bone of the metaphysis,[9] the smallest amount with the compact bone of the shaft. In each case the radioactivity appears to be proportional to the endosteal surfaces rather than to the mass of calcified bone tissue.

There is no comparable effect of endosteal bone formation from administration of estrogenic hormones in any other species of laboratory animals studied thus far.[10] There is a superficially similar response in the rat,[11, 12] but this effect can be observed only during the period of active growth. In the rat, the action is confined to a specific effect upon one particular part of the growth apparatus, that is, interference with resorption or removal of the spongy bone in the metaphysis. Estrogens do not produce any changes in the teeth, alveolar process, gingival epithelium, or the epithelial attachment.[5, 6] As in the mouse, there is no change in the calcium content of the serum during the bone changes.

The physiologic significance of the effect in birds is clear. Medullary bone provides a reservoir of calcium readily available for calcification of the eggshell at the time it is needed. No such need exists in the mouse or in the rat, and not even a teleological explanation of the phenomena observed in these animals is forthcoming.

In man, hormonal influences in skeletal tissue are complex and are as yet not fully clarified. The current widely-held belief[13] that osteoporosis in the female occurs as a result of deprivation of ovarian hormone following either natural or artificial menopause rests upon the sequence of events as observed clinically and upon the formation of new bone as induced in birds and in mice by administration of estrogens. Osteoporosis is thus attributed to a failure of the organism to produce new bone matrix to replace that lost in the physiologic turnover of skeletal tissue, and this failure is in turn attributed to a deficiency of estrogenic hormone.

In keeping with these beliefs, it is now common practice to treat postmenopausal osteoporosis by the administration of estrogens but, although symptomatic improvement and increased retention of nitrogen, calcium, and phosphate have been observed under this therapy, there is little or no direct evidence of increased density of bone in patients so treated. Moreover, there is no sound basis for extrapolating to man or to other higher animals the effects of estrogens on the bones of birds and of mice.[14] There is thus, at best, only presumptive evidence either that a lack of estrogenic hormones results in failure of new bone formation in man or that administration of these hormones leads to new bone formation after such a failure has occurred.

In view of these considerations, and since the results of estrogen therapy in osteoporosis remain inconclusive even after some years of intensive study, it would seem that the time has come for a new and long look at the entire problem of the relationship of estrogenic hormones to osteoblastic activity and bone formation in man and in other animals higher than birds, mice, and rats.

References

1. Kyes, P. & T. S. Potter. 1934. Physiological marrow ossification in female pigeons. Anat. Record. **60:** 377–379.
2. Bloom, W., M. A. Bloom & F. C. McLean. 1941. Calcification and ossification. Medullary bone changes in the reproductive cycle of female pigeons. Anat. Record. **81:** 443–475.
3. Gardner, W. U. & C. A. Pfeiffer. 1938. Skeletal changes in mice receiving estrogens. Proc. Soc. Exptl. Biol. Med. **37:** 678–679.
4. Urist, M. R., A. M. Budy & F. C. McLean. 1950. Endosteal-bone formation in estrogen-treated mice. J. Bone and Joint Surgery. **32A:** 143–162.
5. Stahl, S. S., J. P. Weinmann, I. Schour & A. M. Budy. 1950. The effect of estrogen on the alveolar bone and teeth of mice and rats. Anat. Record. **107:** 21–41.
6. Nutlay, A. G., S. N. Bhaskar, J. P. Weinmann & A. M. Budy. 1954. The effect of estrogen on the gingiva and alveolar bone of molars in rats and mice. J. Dental Research. **33:** 115–127.
7. Budy, A. M. 1955. Metabolism, excretion, and retention of C^{14} labeled estrone in immature mice. Arch. intern. pharmacodynamie. **103:** 435–452.

8. Budy, A. M. 1954. Metabolism, excretion, and retention of radioactive estrone-16-C^{14} in immature mice. Thesis. Univ. Chicago. Chicago, Ill.
9. Budy, A. M. 1956. Metabolism and distribution of C^{14} labeled estrone in immature mice. J. Pharmacol. Exptl. Therap. **116**: 10.
10. Urist, M. R., A. M. Budy & F. C. McLean. 1948. Species differences in the reaction of the mammalian skeleton to estrogens. Proc. Soc. Exptl. Biol. Med. **68**: 324–326.
11. Day, H. G. & R. H. Follis, Jr. 1941. Skeletal changes in rats receiving estradiol benzoate as indicated by histological studies and determinations of bone ash, serum calcium and phosphatase. Endocrinology. **28**: 83–93.
12. Budy, A. M., M. R. Urist & F. C. McLean. 1952. The effect of estrogens on the growth apparatus of the bones of immature rats. Am. J. Pathol. **28**: 1143–1167.
13. Albright, F. & E. C. Reifenstein, Jr. 1948. The Parathyroid Glands and Metabolic Bone Disease. Selected studies. Williams & Wilkins. Baltimore, Md.
14. McLean, F. C. & M. R. Urist. 1955. Bone: An Introduction to the Physiology of Skeletal Tissue. : 152–155. Univ. Chicago Press. Chicago, Ill.

CLINICAL FEATURES OF IDIOPATHIC HYPOPARATHYROIDISM IN CHILDREN

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The influence of the parathyroid hormone on certain aspects of calcium and phosphorus metabolism is clearly established. It is generally held that the hormone has a dual action involving both the intestinal absorption of calcium and the urinary excretion of phosphorus, though a distinct possibility exists for a direct action on bone.[1]

The genesis of the classic symptom of parathyroid insufficiency, namely, tetany, follows naturally from a consideration of these factors. The symptomatology of the hypoparathyroid state, however, is not a simple one. Patients who suffer from idiopathic hypoparathyroidism may present an array of symptoms and signs so bizarre that the true diagnosis remains unsuspected for long periods and, even though the diagnosis be made and treatment instituted, complications that are in no conceivable way connected with the known actions of the hormone may appear further to confound the clinician. It is the purpose of this essay to discuss the clinical features of this rare condition in the hope that a fuller understanding of the intricacies of parathyroid hormone function may be possible in the future. To this end, 7 hitherto-unreported cases of idiopathic hypoparathyroidism in children will be reported, and the pertinent literature will be reviewed. Six of these cases fulfill all of the usual diagnostic criteria for this condition, while the seventh can best be classed as a probable case.

Excellent reviews of the subject are to be found in the papers of Lachmann,[2] Jordan and Kelsall,[3] and Steinberg and Waldron.[4] The salient diagnostic features of this disorder include symptoms of tetany or tetanic equivalents, a reduction in the level of serum calcium and an elevation in serum phosphorus* in the absence of significant disease of the genitourinary and gastrointestinal tracts, and an increase in the level of serum calcium and the urinary phosphorus excretion upon administration of parathyroid hormone.

The etiology of the idiopathic or "spontaneous" variety of hypoparathyroidism, in contrast to the postoperative or neonatal forms, is but poorly understood. The few autopsies that have been done have revealed either an absence of the glands or their replacement by fatty tissue. It is generally believed that either a congenital absence of the glands or degeneration subsequent to trauma or infection are the most likely possibilities, and a recent report would indicate that maternal hyperparathyroidism may also have to be considered in view of its role in the pathogenesis of prolonged neonatal tetany.[5] A genetic factor is indicated by the occasional occurrence of the disease in more than one member of a sibship. The disease is rare and, in the majority of instances, symptoms become manifest during childhood.

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* Steinberg and Waldron's requirement of a serum phosphorus of greater than 7 mg. per cent for children may be too strict, since 2 of our cases (IV and VI) in whom there would seem to be no doubt as to the diagnosis did not fulfill this criterion.

The cases to be presented are numbered in the order in which they were seen by the author. Only those data pertinent to the hypoparathyroid state will be presented here. Complete protocols are recorded in the Appendix.

Symptomatology at Onset

In case I, the early symptoms consisted of blepharitis, photophobia, corneal scarring, and irritability, leading to tentative diagnosis of allergy. Later the clinical picture was dominated by convulsions, some of which were typically *grand mal* in type, others questionably tetanic. The patient was considered to have idiopathic epilepsy, and it was not until another year had passed that the observation of definite carpopedal spasm led to the establishment of the diagnosis of hypoparathyroidism. It was not until after treatment had been started that symptoms of adrenocortical insufficiency appeared. The patient is currently being treated for both conditions.

In case II, brittleness of the hair and dryness of the skin was noted at a very early age, and thyroid medication was given for a prolonged period on the supposition that a state of hypothyroidism existed. Later the patient began to have generalized convulsions, and although there is no clear description of tetany, calcium therapy was tried, and the prompt and satisfactory clinical response to it in the face of a lack of effect from the usual anticonvulsants led to the diagnosis. Alopecia developed despite the use of parathormone and calcium in amounts sufficient to control the convulsions completely. It is of interest that a series of 2 convulsions, associated with a febrile illness, occurred during infancy. This case must be classed as probable in view of the available information (see protocol in Appendix).

In case III, deepening of the voice was noted in early infancy; at the age of 18 months the patient had a series of tonic convulsions; at 3 years of age marked irritability and dental caries were noted. Shortly afterwards, the occurrence of attacks of dyspnea, stumbling, muscle rigidity, and carpopedal spasm prompted the institution of calcium therapy. Subsequently, brittleness and partial loss of hair occurred, and the patient failed to grow normally. About six years after the first episode of tetany, definitive treatment for the hypoparathyroid state was finally instituted. Hypoadrenocorticism supervened eventually to cause death at the age of 14 years. At autopsy, the left adrenal could not be found, and the right was markedly atrophic. The parathyroid glands were absent.

Case IV had hypocalcemia and convulsions as a newborn infant. Whether he was suffering from the entity known as "temporary hypoparathyroidism of the newborn" can only be surmised. At any rate, the principal symptoms during the 7 years prior to the establishment of the diagnosis consisted of head nodding, nystagmus, and questionable mental retardation. Eventually a presumptive diagnosis of cerebral palsy was made. Late in childhood, generalized tonic-clonic convulsions associated with carpopedal spasm were noted. A history of intracranial birth hemorrhage makes it difficult to be certain that all of this patient's symptoms can be assigned to lack of parathyroid hormone. There has been, however, marked improvement with treatment.

Case V was noted to have a deep voice as an infant. She later developed tetanic equivalents characterized by facial cyanosis and grimacing, stumbling, and muscle cramps. Still later she developed generalized tonic-clonic convulsions accompanied by laryngeal stridor and postictal spasticity. She was thought to be suffering from epilepsy, and anticonvulsants were given (without effect) for 1½ years before a diagnosis of hypoparathyroidism was made.

Case VI, a sibling of case V, was born 2 years after his sister's diagnosis was made. It is perhaps of signifance that he had been given approximately 2500 units of vitamin D daily since early infancy. The occurrence of cramps and spasm of the calf muscles prompted an examination by a physician who, keeping in mind the sister's disease, elicited Chvostek's sign and immediately took steps to investigate the possibility of hypoparathyroidism.

In case VII, the early clinical picture was dominated by moniliasis involving the skin, nails, mouth, and larynx. Later, partial loss of hair occurred, followed by dental caries, intention tremor of the hands, abdominal pain, and severe attacks of laryngeal stridor, wheezing, dyspnea, and vomiting that were considered to be asthmatic in character. Shortly following the institution of treatment for the hypoparathyroid state, total alopecia developed.

These patients serve to emphasize the variation in clinical mode of onset of idiopathic hypoparathyroidism. In all but one there was a considerable time lapse between the onset of symptoms and establishment of the diagnosis. The reason for this becomes clearer from a consideration of the initial complaints in which the pathognomonic symptom of tetany was so frequently adumbrated by tetanic equivalents and ectodermal, or "trophic," disturbances. The authors of a recent review have calculated that, for cases reported in the literature, an average of 8 years elapsed between onset of symptoms and the institution of therapy.[1] In the experience of others, the initial predominating symptom or symptoms have been variously recorded as photophobia and ectodermal dysplasia,[6] alopecia,[7] leg pain, chronic moniliasis, and *grand mal* convulsions,[8] convulsions and adrenal insufficiency,[9] convulsions, muscle cramps, and increased intracranial pressure,[10] paresthesias, excessive dental caries and, later, carpopedal spasm in a patient who was symptomatic for 31 years before the diagnosis was made.[1] It is evident that great variability exists from patient to patient with regard to the form taken by the ectodermal lesions and the tetanic equivalents. *Grand mal* epilepsy was the most common misdiagnosis among the collected cases.[4]

A moment's reflection should convince one of the difficulties faced by the clinician in this area. Extensor spasm of the hands and feet, mimicking true carpopedal spasm, can occur in the absence of hypocalcemia or alkalosis as a manifestation of central nervous system disease. An insidious onset, coupled with a bizarre symptomatology as manifestations of a rare disease cannot fail to tax the ingenuity of even the most astute diagnostician.

Summary of Findings

The pertinent data in regard to our patients are summarized in TABLE 1. It is impossible, of course, to state exactly when the hypoparathyroid state became established in all the patients, and some may object to the inclusion of the early

TABLE 1

Case number	I	II	III	IV	V	VI	VII
(1) Central nervous system							
Convulsions	+	+	+	+	+		
Abnormal EEG	+	+	+	+	+	+	
Intracranial calcification			+				
Mental retardation (?)				+			
(2) Respiratory tract							
Laryngeal stridor					+		+
Deep voice			+		+		+
Dyspnea			+				+
(3) Moniliasis							+
(4) Peripheral neuromotor system							
Tetany	+	?	+	+	+	+	
Muscle pain or cramps	+				+	+	+
Awkwardness			+	+	+		
Tremor				+			+
(5) Skeleton							
Increased density			+				
(6) Gastrointestinal tract							
Vomiting, abdominal pain	+						+
(7) Ectodermal structures							
Skin, hair, nails		+	+		+		+
Conjunctiva	+						
Cataracts	+		+				
Teeth			+		+	+	+
(8) Adrenal hypofunction	+		+				

symptoms as an integral part of the disease. Nevertheless, many of these disturbances have been known to occur subsequent to the establishment of the diagnosis, some have been described in postoperative hypoparathyroidism, and a similar spectrum of symptomatology has been described by a number of observers. Consequently, it would appear justifiable to include the earliest manifestations, as set forth in our cases, in the symptomatology.

It is our contention that the diagnosis of hypoparathyroidism has been satisfactorily established in 6 of the cases, and is probable in the other (case II). None shows evidence of chronic renal or gastrointestinal insufficiency or of rickets. In 5 of them, the administration of parathyroid hormone resulted in a prompt phosphorus diuresis (FIGURE 1). None show the physical or roentgenographic attributes of "pseudohypoparathyroidism."[1]

As far as case II is concerned, the weakest link in the diagnostic evidence is the normal value for serum phosphorus. The only values available were obtained at a time shortly after the discontinuance of parathormone therapy and after a prolonged period on an almost milk-free diet. The referring physician stated that the pretreatment serum calcium and phosphorus levels were diagnostic. The rapid response to parathormone therapy, the low levels of urinary calcium and phosphorus, and the absence of other causative factors would tend to support the diagnosis.

For purposes of discussion, the findings in spontaneous hypoparathyroidism[1-4, 9] can be grouped in various categories:

(1) *Central nervous system phenomena*, such as increased cerebrospinal fluid pressure (including papilledema), intracranial calcification, psychotic behavior,

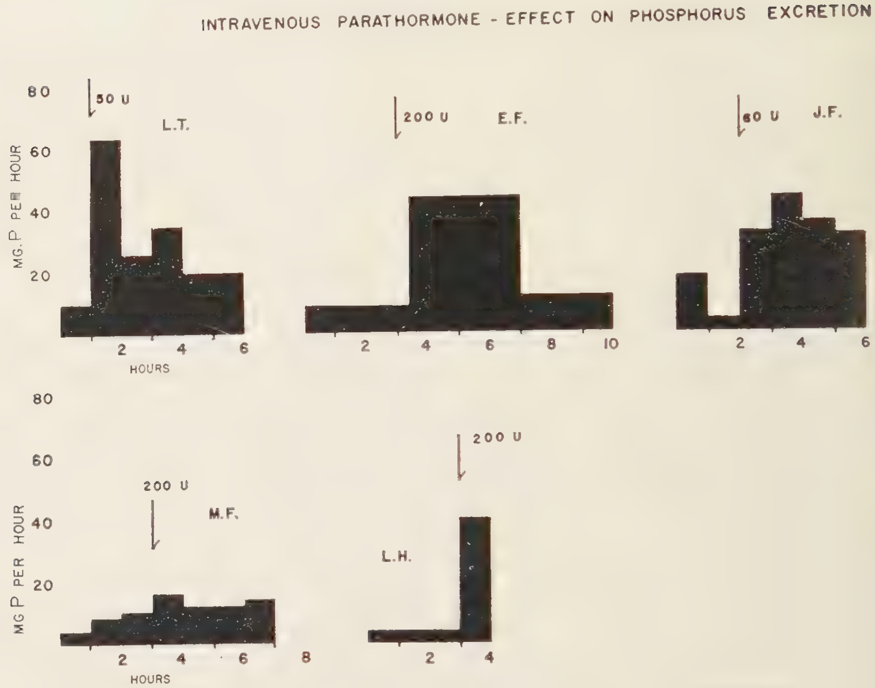

FIGURE 1. Effect of intravenous parathyroid hormone on urinary excretion of phosphorus.

mental retardation, epileptiform convulsions, and electroencephalographic abnormalities. It cannot be too strongly emphasized that the convulsions can be of the classical *grand mal* variety, to the point of incontinence and postictal drowsiness. Cerebral dysrhythmia was noted in 6 of our patients (no record was taken in case VII). In 4, paroxysmal bursts were also recorded. The use of such anticonvulsants as phenobarbital and dilantin has been ineffectual in our cases.

FIGURE 2 illustrates intracranial calcification in case III. There is some disagreement as to whether soft-tissue calcification occurs in areas other than the brain, Talbot stating that it is common[9] and McGregor maintaining that it has never been reported.[31] None could be detected in our patients. Metastatic calcification has been reported, however, in pseudohypoparathyroidism.

(2) *Respiratory tract symptoms* in the form of hoarseness, laryngospasm, wheezing, and dyspnea. It is of interest that in 3 of our patients the voice deepened in pitch at an early age, and in only 1 of them (case VII) can this be said to be due to laryngeal moniliasis. This symptom has persisted throughout the follow up period in each instance. As noted above, 1 of our patients was thought to have allergic asthma at 1 point in the course of her illness.

(3) *Cutaneous and membranous moniliasis.* The basis for the frequently observed association between this fungus infection and hypoparathyroidism is most obscure. The infection is often chronic and, as in case VII, may cause

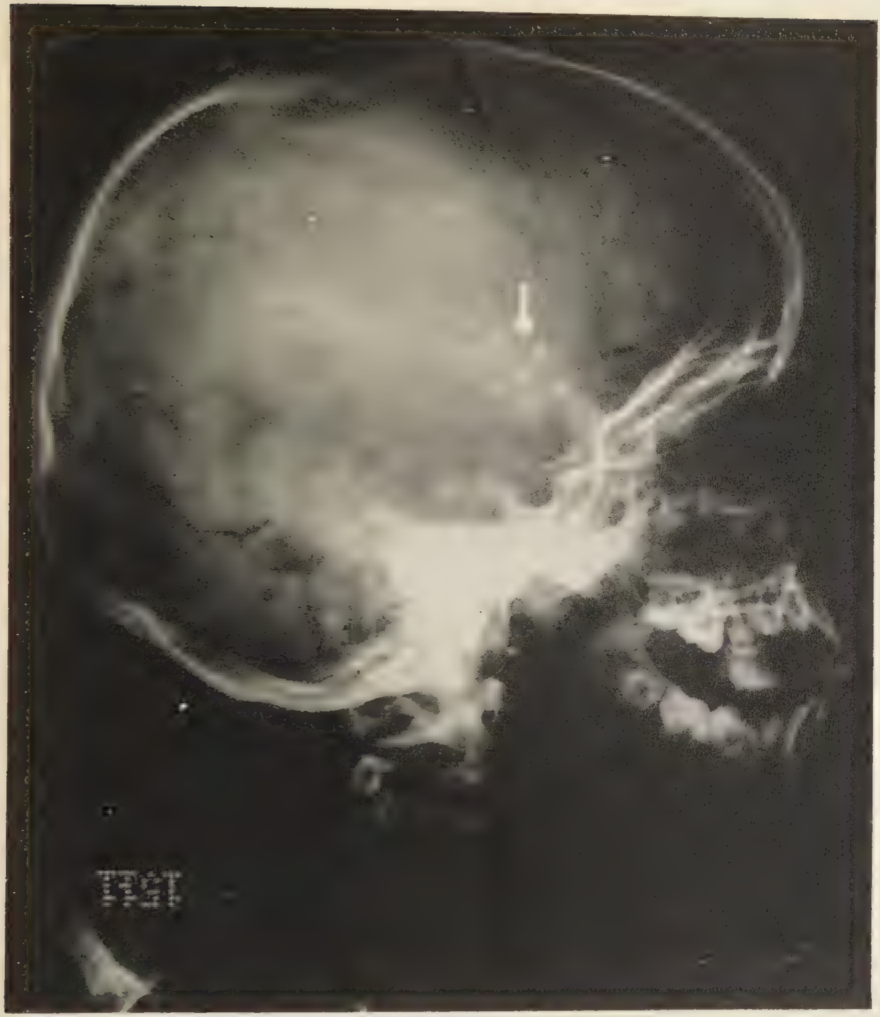

FIGURE 2. Skull roentgenogram showing intracranial calcification in case III

troublesome symptoms.[9-11] FIGURE 3 illustrates the involvement of the fingernails in case VII.

(4) *Peripheral neuromotor abnormalities*, such as paresthesias, muscle rigidity, muscle cramps and pain, and tetany. The muscle rigidity may sometimes lead to gait disturbances, as in cases III and V. Abdominal pain can be quite distressing and could conceivably cause difficulties in diagnosis. Facial grimacing occurred in one of our patients (case V). In our experience, Chvostek's sign is more reliable than Trousseau's and, with a little practice, the 3 divisions of the facial nerve can be activated separately.

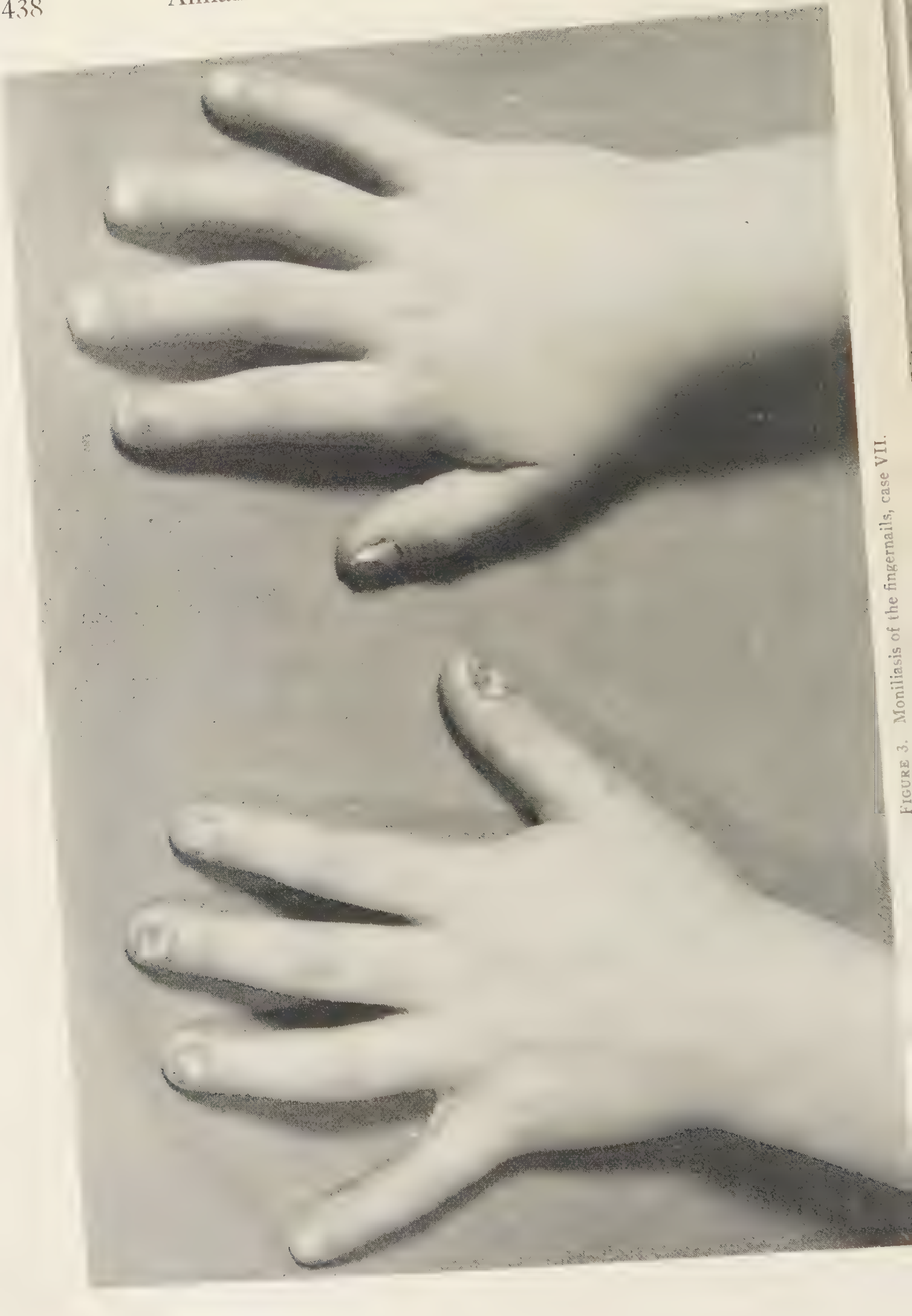

FIGURE 3. Moniliasis of the fingernails, case VII.

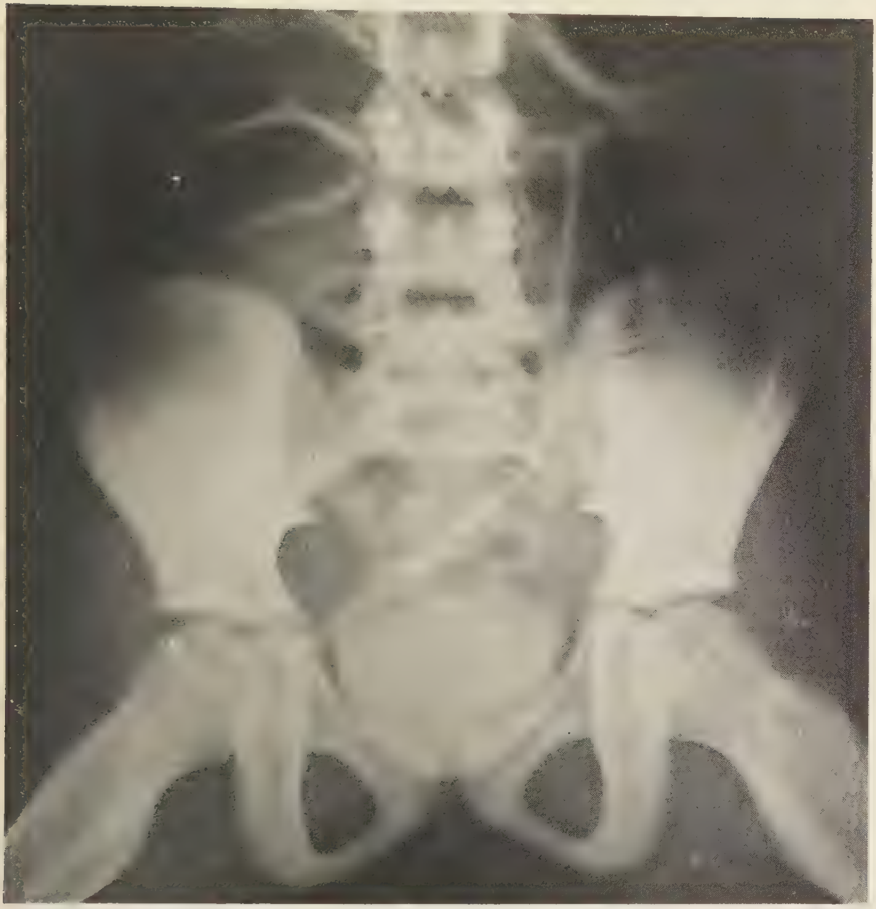

FIGURE 4. Pelvic roentgenogram showing increased bone density. Radio-opaque dye present in bladder.

(5) *Increased roentgenographic density of the skeletal structures.* This was clearly demonstrated in 1 of our cases (case III, FIGURE 4), but could not be definitely established in the others. It is of interest in this connection that the mother of patient III volunteered the information that she craved milk and puddings for a number of years. Some recession in skeletal density was noted after 2 years of treatment.

In cases III and V the lamina dura was definitely increased in density and in width (FIGURE 5), a finding that one would expect off-hand, since the opposite change is characteristic of hyperparathyroidism. To my knowledge, this has not been reported heretofore. Another finding in case V was the presence of calcification in the patient's dental pulp, an abnormality also present in her mother's teeth, but not in those of her brother. This latter finding may well be incidental, though Jordan and Kelsall's patient also had pulp stones.[3]

(6) *Gastrointestinal symptoms*, most commonly abdominal pain and vomiting.

(7) *A variety of disturbances of ectodermal structures*, namely,

(a) Dryness of the skin, sufficiently pronounced in case II to be considered consistent with hypothyroidism. A variety of dermatides have been described in the older literature.[2]

(b) Changes in the hair, consisting of either brittleness and thinning or alopecia, are fairly common, and were noted in 4 of our cases. FIGURES 6 and 7 illustrate the extent of loss of hair in cases II and VII. It is of interest that in each, alopecia developed after the institution of treatment, this being parathormone in case II and vitamin D in case VII. In the latter instance, hair also disappeared from the eyebrows and the extremities. A.T. 10 therapy was substituted for several months without observable change. After about 1 year of total alopecia, and at a time when she was again taking vitamin D, scalp and body hair began to reappear and at present are almost normal. Case II, now in her fourth year of treatment, remains completely bald.

(c) Ridging, pitting, brittleness, and loss of the fingernails and toenails.

(d) Chronic conjunctivitis and photophobia.

(e) Optic cataracts that are usually small but may reach sufficient size to interfere with vision.

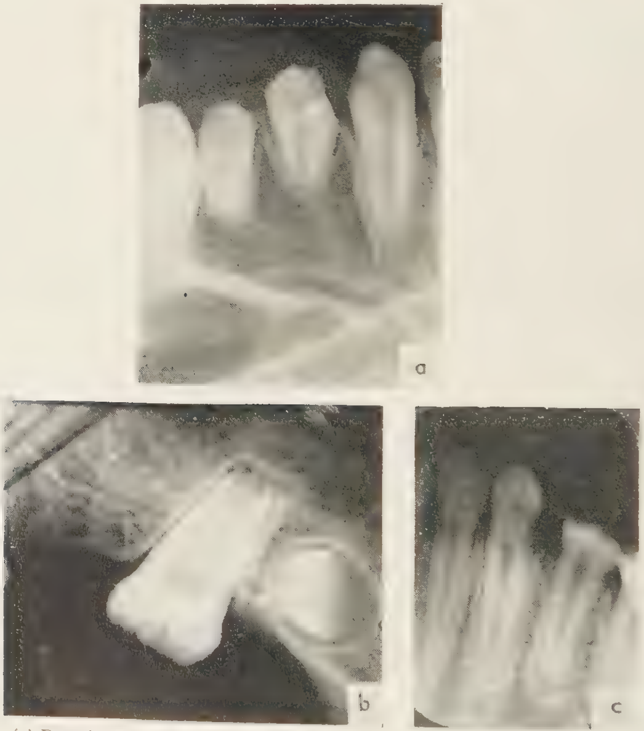

FIGURE 5. (a) Dental roentgenogram showing changes in lamina dura and enamel, case III; (5b) and (5c) dental roentgenograms showing enamel hypoplasia, thickening of the lamina dura, and pulp calcification, case V

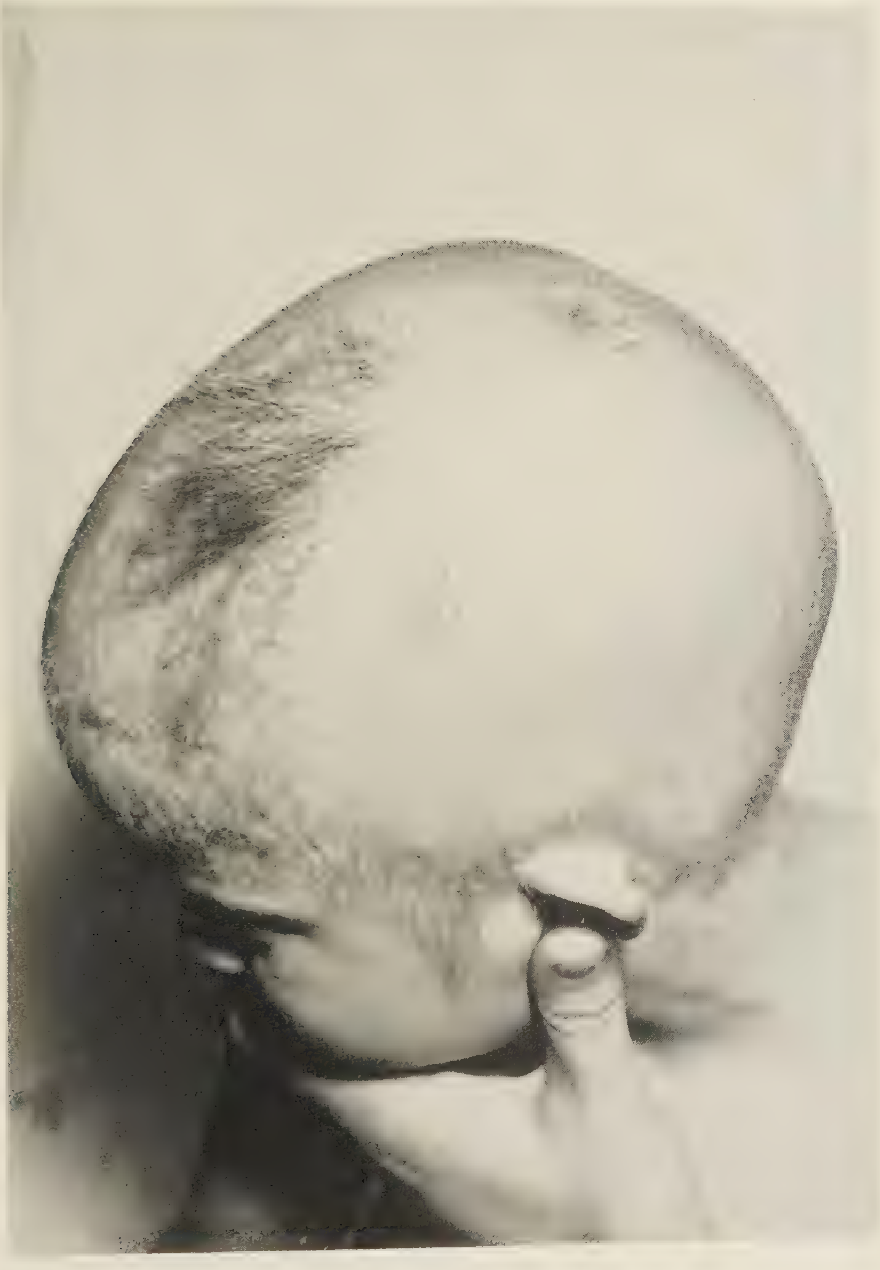

Figure 6. Appearance of scalp in case II.

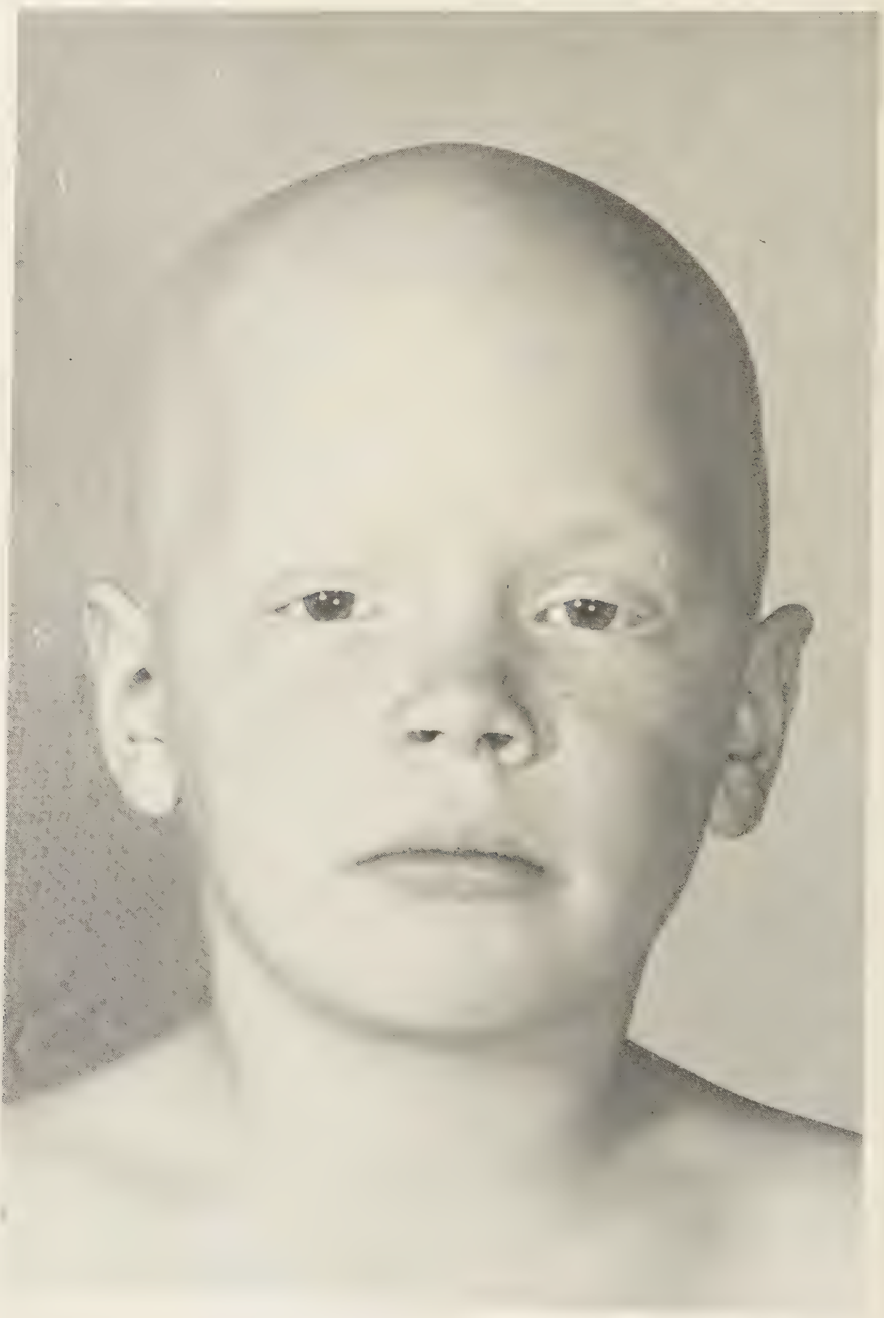

FIGURE 7. Appearance of face and scalp in case VII.

(f) Dental abnormalities such as caries, premature loss of teeth, and enamel hypoplasia. The finding of an increase in width of the lamina dura in 2 of our patients (FIGURE 5) has been alluded to above. Jordan and Kelsall's patient had delayed eruption of teeth,[3] and Lachmann mentions that most of his patients have lost their teeth.[2] The dental lesions have best been described in experimental deficiency,[12] with credit to J. Erdheim (1906) for being the first to associate these and other trophic lesions with parathyroid insufficiency.

(8) *Adrenocortical insufficiency* is a rare complication, with only 6 recorded cases[7, 13] in addition to the 2 patients in the present series (cases I and III). Case I, in which the diagnosis is admittedly based on clinical grounds alone, is doing well under therapy. Case III expired suddenly in what was probably an adrenal crisis. Marked atrophy of both cortical and medullary elements was noted on histologic examination. An additional case of Addison's disease in a child who had chronic tetany and intracranial calcification was reported a number of years ago,[14] though unfortunately no study of the parathyroids was made.

A point of considerable interest concerns the effect of the Addisonian state upon the manifestations of hypoparathyroidism. It is principally with this point in mind that the course of case III is presented diagrammatically in FIGURE 8. There was a definite tendency for the level of serum calcium to be higher during the period when Addisonian symptoms were most profound, the high levels persisting during a 4-week period of discontinuance of calcium and vitamin D therapy while various adrenal function tests were underway. The tendency for the level to fall with adrenal substitution therapy is also evident. This phenomenon has also been described by Papadatos and Klein[7] and by Leifer and Hollander.[13] A depression of serum calcium has also been observed in a patient with hypoparathyroidism who was under treatment with cortisone for concurrent rheumatoid arthritis.[15]

Surveys of adrenocortical function have been carried out in most of the other patients in this series, with negative results, as follows: normal corticotropin-eosinophile tests in cases II, IV, V, VI; normal response to water loading (case VII); urinary 17-ketosteroids of 5.9 mg. 24 hours in case V (age 13), 1.7 mg./24 hours in case VI (age 9), and 0 in case VII (age 6); and normal values for serum Na, K, CO_2, and Cl (cases IV, V, VII).

The nature of the relationship between the parathyroid and the adrenals is obscure. One instance of familial hypoparathyroidism (3 affected sibs) has been described, in which a paternal aunt, and possibly the paternal grandfather, had Addison's disease.[16] A hypoparathyroid patient whose sibling had Addison's disease has also been reported.[17] Sutphin *et al.*[11] have reported 3 affected sibs born of a consanguineous marriage, with coexistent Addison's disease in 1. Of even greater interest is the recent paper of Craig *et al.*,[18] describing 3 cases of Addison's disease associated with chronic moniliasis. Ectodermal changes were manifest in all 3. At autopsy no parathyroid tissue could be found in 1 of the patients and, in another, only a small remnant of a gland was present. Despite these findings, neither patient manifested tetany, clear cut tetanic equivalents, or diagnostic changes in serum calcium and phosphorus levels. A sibling of 1 of these patients had chronic moniliasis and clinical hypoparathyroidism.

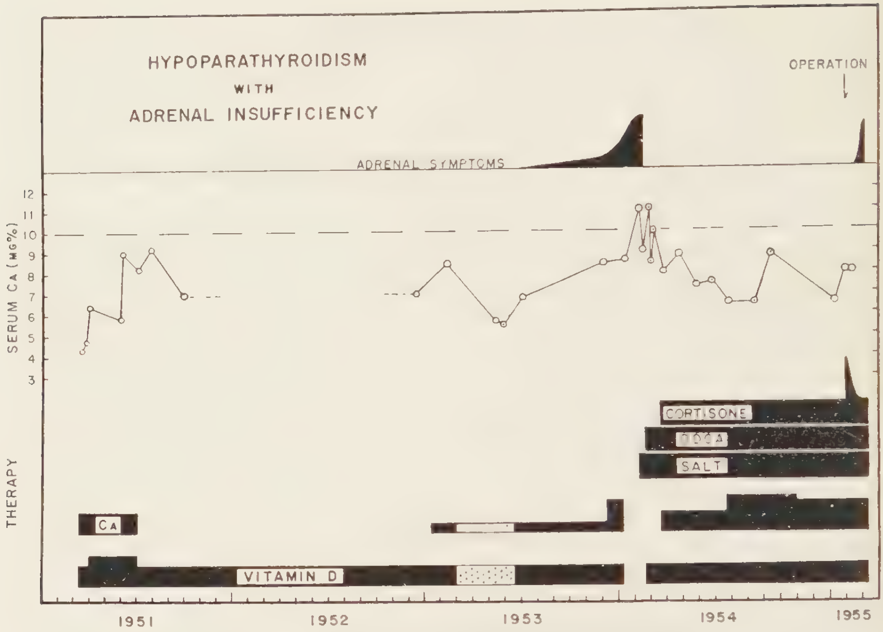

FIGURE 8. Certain aspects of the clinical course in case III.

It is thus difficult to escape the conclusion that a genetic factor underlies the relationship between these 2 endocrine organs.

In addition to the long list of associated disturbances categorized above, a case has recently been described which exhibited skeletal demineralization and cardiac failure, the latter supposedly the result of extreme hypocalcemia.[19] One of our cases (VII), however, failed to manifest signs of cardiac failure in the face of an equally profound depression in serum calcium (3.6 mg. per cent). In another recently recorded case, concurrent pernicious anemia was noted, though no claim was made for causal interrelationship.[20] An instance of questionable hypothyroidism has also been reported.[21]

Discussion

The clinical picture of idiopathic hypoparathyroidism is both confusing and fascinating. In an attempt to explain the pathogenesis of the observed clinical features, several possibilities should be considered.

First, could not the nontetanic manifestations merely be the result of chronic exposure of body tissues to a low-calcium, high-phosphorus medium? Disturbances of the skin, hair, and nails have been noted in a few patients with chronic steatorrhea,[22] but here the possibilities of associated nutritional deficiencies are real. Furthermore, the level of serum phosphorus is usually not elevated. The only other chronic disease commonly exhibiting a low-calcium, high-phosphorus level is renal insufficiency and, to my knowledge, typical trophic lesions do not occur here. Perhaps the strongest point against this hypothesis is the occurrence of trophic changes prior to the onset of tetany.

Second, is there a genetic factor, or 1 or more associated factors, responsible for both the integrity of ectodermal structures and elaboration of the hormone, and perhaps the adrenal as well? In support of this is the familial association between hypoparathyroidism and hypoadrenocorticism, and what would appear to be a high incidence of dental disease in the families of some of our patients. There can be little doubt that genetic factors are operative in some cases of idiopathic hypoparathyroidism, and it would be possible to postulate that variations in the clinical picture merely reflect variations in genetic expressivity. The clinical manifestations seen in postoperative hypoparathyroidism, however, argue against such an hypothesis.

Third, as Lachmann has stated, there is evidence to "suggest the hypothesis that the parathyroids may have two functions, namely, regulation of the blood calcium concentration and another function, the failure of which gives rise to the trophic and possibly also the nervous disturbances." The occurrence of ectodermal disturbances in parathyroidectomized animals, the lack of correlation between ectodermal symptoms and the level of serum calcium and/or tetany in some human patients, and the report of Craig *et al.*[18] of trophic changes without clear-cut hypocalcemia in 2 aparathyrotic patients can all be offered in support of this hypothesis. On the other hand, treatment with vitamin D and A.T. 10 (neither of which are considered to possess any semblance of the postulated "trophic" factor of the parathyroid hormone) will forestall the occurrence of trophic changes in some patients and effect their repair in others. Perhaps a subdivision of ectodermal or trophic disturbances (since adrenal cortex is of mesodermal origin) should be made in which those most closely associated with hypocalcemia (such as cataract) are separated from those in which the association is least evident, namely, adrenal insufficiency and moniliasis.

Summary

The clinical spectrum of idiopathic hypoparathyroidism is presented, with the help of data from the literature and from the author's experience with 7 cases (6 typical, 1 probable).

In addition to tetany, these patients suffered from a variety of symptoms and signs involving ectodermal and central nervous system structures. Two patients in the present series had associated Addison's disease, and 1 suffered from widespread moniliasis.

A new finding is described, namely, an increase in the width of the lamina dura.

Acknowledgments

Determination of urinary 17-ketosteroids were made in E. H. Keutmann's laboratory, Department of Medicine, University of Rochester, Rochester, N. Y.

Dental examinations were made by R. R. Hawes, Eastman Dental Dispensary, Rochester, N. Y.

Case I is being published with the permission of E. M. Wier, of Fort Worth, Texas, and E. L. Pratt, Dallas, Texas. Wier very kindly provided date from his own records. Hospitalization was at the W. I. Cook Memorial Hospital

Center for Children, Fort Worth, and the Texas Children's Hospital, Dallas, Texas.

Case II is published with the permission of Forest Cornwell, Dallas, Texas, who kindly provided data from his records. Hospitalization was at the Texas Children's Hospital in that city.

The remaining cases were seen at the Strong Memorial Hospital, Rochester, N. Y.

Case III is to be reported in greater detail by James Drorbaugh and Robert Beilman. J. Tannenberg, of Batavia, N. Y., performed the autopsy, and I am indebted to him for permission to include the autopsy findings.

APPENDIX

Case I. LaJuan H., a white female born November 11, 1946, enjoyed normal infancy except for an episode of mild pertussis at the age of 4 months. At the age of 21 months, reddening and itching of the conjunctivae developed, and what were variously described as phlyctenules and corneal ulcerations were noted. There was no response to various local medications and, by the age of $3\frac{1}{2}$ years, the symptoms had progressed to include severe photophobia, blepharitis, and bilateral corneal opacities. Marked irritability was also a frequent complaint. Local instillations of cortisone provided some relief.

At the age of $4\frac{1}{2}$ years about 2 months after an attack of rubeola, the patient began to have episodes described by the mother as "fainting spells," questionable carpopedal spasm with generalized muscle rigidity and opisthotonus, and generalized tonic-clonic convulsions associated with loss of consciousness, movement of the eyes upward and to the right, urinary incontinence, and postictal somnolence. Frequent episodes of abdominal pain were also described.

Investigation at that time revealed the presence of bilateral chronic keratitis and mild ataxia, but no other neurologic findings. A pneumoencephalogram revealed no abnormalities. Generalized dysrhythmia was recorded on the electroencephalogram. A diagnosis of idiopathic epilepsy was made, and treatment with dilantin and phenobarbital begun.

During the next year 3 more convulsions occurred. At the age of $5\frac{1}{2}$ years hospitalization again became necessary due to an episode of vomiting, abdominal pain, and prolonged semidelirium. Observation of a tetanic convulsion at this time led to further study culminating in the diagnosis of hypoparathyroidism. The serum calcium value was 4.5 to 5.0 mg. per cent and the serum phosphorus 11.9 to 13.0 mg. per cent. Treatment with A.T. 10, added calcium, and aluminum hydroxide produced rapid improvement, with an increase in the serum calcium value to the 8 to 9.8 mg. per cent range and a decrease in serum phosphorus to 6.4 mg. per cent.

During the next 2 months she had 1 further convulsion. She then began to have intermittent cramping abdominal pain, anorexia, apathy, weakness, and weight loss. She was again examined in a hospital. There were no signs of excessive weight loss or dehydration. The skin was quite dry. There were no signs of tetany. Studies included an observation of the response to the intravenous administration of 200 units of parathyroid hormone (FIGURE 1). Circulating eosinophiles fell to 73 per cent of the fasting level in response to epinephrine.

Following discharge from the hospital, her gradual downhill course continued. At the end of 3 months she developed nausea and vomiting, rapidly became dehydrated, and was readmitted to a hospital in Addisonian crisis. Examination revealed evidences of profound dehydration, pallor, drowsiness, and mild hypotension (blood pressure 88/60 as compared to subsequent readings of 100/65). No signs of tetany could be elicited, and there was no evidence of hyperpigmentation of the skin. By this time the evidences of blepharitis and keratitis had practically disappeared, though the corneal opacities remained. Several small anterior cataracts were now visible in each lens. During the preceding 5 months, she had lost about 16 lb. in weight.

Large quantities of fluids were given parenterally in an attempt to achieve a better state of hydration, but she continued to vomit and to be anorexic. Unfortunately, no studies of the serum electrolyte concentrations could be done at this time. Because of the persistence of symptoms, however, a clinical diagnosis of adrenocortical insufficiency was made, an impression confirmed by the prompt, gratifying, and sustained response of the patient to the administration of cortisone, DOCA, and sodium chloride.

During the ensuing 3 years (the extent of the present follow-up), the patient has been under rather good control: she has grown normally; her eyes have improved to the point where the conjunctivae are now normal, though one small corneal opacity remains (the status of the cataracts is unknown); and she has weathered several intercurrent febrile illnesses. Shortly following the institution of adrenal substitution therapy, 3 tetanic convulsions occurred, but there have been no convulsions since. Therapy has consisted of A.T. 10 (0.5 cc. 2 or 3 times weekly), calcium lactate (10 gm. daily), cortisone (25 mg. daily by mouth), DOCA (½ mg. daily sublingually), and sodium chloride (2 gm. daily). About 6 months ago, hydrocortisone (5 mg. daily) was substituted for cortisone, and the DOCA discontinued.

Other pertinent studies carried out during the course of her illness included: normal roentgenograms of the skull and long bones; cerebrospinal fluid pressures of 140 mm. and 160 mm. (the latter reading obtained when tetany was severe); repeated normal urinalyses and peripheral blood counts; normal values for serum protein and nonprotein, nitrogen; normal values for serum Na, Cl, K and CO_2 content (after adrenal substitution therapy was underway); negative tuberculin test; and normal funduscopic examinations. Unfortunately, cultures for monilia have not been done.

There is no history of a similar disorder in the family.

Case II. Maria F., a white female (birth date December 19, 1947), was first seen at the age of 4 years. She was born prematurely, weighing 4 lbs. 11 oz., after a prolonged labor. There was a tendency to vomit feedings frequently in early infancy. At 3 months of age her hair became brittle and would grow no longer than ½ to 1 inch before breaking off. Her general development was normal except for delayed closure of the anterior cranial fontanelle. At the age of 2 years she had pneumonia complicated by 2 convulsions. At about this time a tentative diagnosis of hypothyroidism was made. Thyroid medication was prescribed, and continued for the next 1½ years without appreciable effect. She has taken very little milk since that time.

At the age of 3½ years she began to have generalized convulsions. Each episode lasted about 1 minute, and was characterized by clonic movements of the extremities, clenching of the fists, twitching of the left eyelid, loss of consciousness, and brief postictal weakness. These occurred at the rate of 20 to 30 per day despite the administration of dilantin and phenobarbital, but ceased promptly when a calcium preparation was given intravenously. The exact levels of calcium and phosphorus in the blood serum are not known. Thyroid therapy was stopped.

Treatment with an oral calcium preparation and thrice-weekly injections of parathyroid hormone was started, and the patient remained asymptomatic until about a week prior to admission. At this time she began to lose her hair in large amounts. All treatment was stopped, and she was referred to the hospital for further evaluation.

There is 1 normal sibling. The paternal grandmother and 4 of her siblings have diabetes. No one in the family has difficulties resembling those of the patient.

On admission to the hospital she weighed 13.9 kg. and was 79 cm. tall. There was a striking loss of hair from the eyebrow region and the scalp (FIGURE 6). There were no signs of tetany. The skin was dry, and the nails, teeth, and eyes were normal. The liver edge could be felt 1½ cm. below the right costal margin. There were no other physical findings of note.

Laboratory examination revealed a normal blood count, negative urinalysis (sp. gr. 1.040), and negative flocculation test for syphilis. The blood urea nitrogen and total serum proteins were normal. The serum calcium was 7.6 mg. per cent and the phosphorus 4.3 mg. per cent; alkaline phosphatase was 10.6 Bodansky units. The 24-hour urinary excretion of calcium was 14 mg. and that of phosphorus 232 mg., and on a subsequent occasion she excreted 45 mg. phosphorus in a 12-hour period. The response in urinary phosphorus excretion to the giving of 200 units of parathyroid hormone intravenously was definite, though not as striking as in our other cases (FIGURE 1). Roentgenograms of the skull and long bones were normal. Electroencephalogram revealed generalized high-voltage slow activity, most pronounced in the right posterior region, but without paroxysms.

Other tests showed a 24-hour radioactive iodine uptake of 20 per cent, a 50 per cent drop in circulating eosinophiles after intramuscular corticotropin, and a normal electrocardiogram.

Treatment with A.T. 10 and calcium was recommended. The only follow-up details for the past 3 years are that her general condition is good, though she remains completely bald.

Case III. Elaine F., a white female, born July 21, 1941, was considered normal for the first 6 months of life. It was at this time that the first deciduous teeth erupted, and these were small, yellow stained, and soft. At 9 months of age she sustained a severe febrile illness of uncertain cause. At 1 year of age her voice was observed to be deep in pitch, but hoarseness and laryngeal stridor is denied, however. At the age of 18 months, without provocation, she had a series of convulsions characterized by sudden fall, rigidity, opisthotonus, and unconsciousness.

At the age of 3 she was admitted to the hospital with a diagnosis of poliomyelitis. Only 18 teeth were present, and many of these were carious. She was extremely irritable. The course of the infection was uneventful, the only residual manifestation being mild weakness of the left leg. A few months later she began to experience episodes of dyspnea, stumbling, muscle rigidity, and carpopedal spasm. These were often precipitated by excitement and overexertion. Treatment with calcium relieved the carpopedal spasm, but the spells of dyspnea and stumbling continued, gradually increasing in frequency. She now began to crave milk and puddings, her hair became brittle and fell out easily, pitting of the nails developed, her growth slowed markedly, and she did poorly in school. Dentition was irregular and her teeth were stained with yellow and white plaques. She often complained of burning on urination. Calcium medication was given irregularly.

There are 3 siblings, 2 of whom suffer from excessive dental decay, but their serum calcium and phosphorus values were found to be normal. Her father had complete dentures by the age of 20, and the paternal grandfather had poor teeth. The mother and several of her female relatives suffer from "pyelitis." There is no history of convulsions, tetany, or Addison's disease in the family.

On examination at the age of 9½ years her weight was 20.3 kg., height 119 cm. (below the third percentile for each). The skin was dry and scaly. Only 14 teeth were present. The nails were brittle and pitted. Chvostek's and Trousseau's signs were positive. Incipient cataracts were seen in both eyes. There was slight thoracic scoliosis and a talipes equinus deformity of the left foot. Intelligence quotient was 90. Serum calcium was 4.0 to 4.8; phosphorus, 5.6 to 7.8; and alkaline phosphatase, 14 Bodansky units. Calcification was seen in the region of basal ganglia on roentgenography, and definite cortical thickening noted for the clavicles, femora, ischia, and vertebral bodies (FIGURES 2 and 4). The electroencephalogram revealed a paroxysmally slow dysrhythmia. Intravenous parathyroid hormone produced a prompt phosphorus diuresis (FIGURE 1). Urinary Sulkowitch reaction was negative.

Other laboratory tests included: normal values for blood nonprotein nitrogen, serum albumin and globulin, serum Na, K, Cl, and CO_2 content; an electrocardiogram consistent with hypocalcemia; normal osseous development on X ray; intravenous and retrograde pyelograms indicated a slight dilatation of the left renal pelvis; and injection of epinephrine produced a 50 per cent drop in circulating eosinophiles.

Subsequent dental roentgenograms revealed enamel hypoplasia and thickening of the lamina dura (FIGURE 5) at the age of 11 years.

Treatment with vitamin D (50,000 units daily) and calcium lactate produced prompt improvement in the patient's symptoms. Her hair became finer in texture, she ceased to have episodes of tetany, and her general condition improved. She did well, except for mild signs of tetany when treatment lapses occurred, for the next 2½ years.

At this time she began to exhibit increasing pigmentation of the skin and a craving for salty foods. Within 3 to 4 months, symptoms of anorexia and weakness appeared, followed within a few weeks by nausea, vomiting, and weight loss.

She was readmitted to the hospital at the age of 12½ years. She appeared chronically ill, mildly dehydrated, and weak. Blood pressure was 100/55. The palmar creases were deeply pigmented. The Chvostek sign was negative. Other physical findings were as described previously.

Laboratory findings included: serum Na 131 mEq./L., K 6.3, CO_2 27, and Cl 90; Ca 11 mg. per cent, P 6.5 mg. per cent. Intravenous corticotropin produced no drop in circulating eosinophiles on 1 occasion, and a 30 per cent drop on another. Urinary 17-ketosteroids were 1.4 mg. per 24 hours. She failed to excrete a water load properly, and the administration of a low-sodium, high-potassium diet produced symptoms of vomiting, marked weakness, and a definite fall in blood pressure within 24 hours. Clinical response to DOCA and salt was prompt and satisfactory.

The patient was subsequently carried on a regime of medication consisting of vitamin D (50,000 units daily), calcium lactate (1.8 gm. daily), cortisone (10 mg. daily), sodium chloride (4 gm. daily), and DOCA (trimethyl acetate form, 25 mg. given once a month intramuscularly). She did quite well for the next year. Evidences of skin pigmentation and cataracts persisted, yet signs of tetany were noted only infrequently. Beginning breast development was first noted at 12 years 10 months of age, and the menarche occurred at the age of 13 ½ years.

For several months she had complained of aching and pain of the left ankle and leg, and a decision was made to correct surgically the pes cavus deformity manifest as a residual from her attack of poliomyelitis some years previously. Prior to operation, her metabolic status was evaluated, and the cortisone dosage increased to 150 mg. daily. The operation was carried out under ether anesthesia, and she tolerated the procedure well. Cortisone dosage was gradually decreased to the former level, and observation in the hospital for the subsequent week failed to reveal any untoward symptoms or signs. She was sent home on the medication regime outlined above.

One week later (on the 19th postoperative day) she complained of weakness, abdominal discomfort and nausea, vomited once, and felt feverish. Her local physician made a diagnosis of "virus infection," and gave her an injection of penicillin. The next morning she suddenly complained of extreme weakness and shortness of breath, and expired.

An autopsy was performed that same day. The findings of interest included cardiac dilatation, massive pulmonary edema, and a generous-sized thymus gland. Careful search failed to disclose parathyroid tissue. The left adrenal could not be identified, and the right one appeared as a paper-thin fibrotic mass measuring 2 × 1 × 0.5 cm. in size. On microscopic section, marked atrophy of the cortex was present, and medullary elements were difficult to identify.

Case IV. Leon T., a white male born March 18, 1946, was sent to a cerebral palsy clinic at 7 years of age because of retarded mental development and chronic muscle tremors. On the fifth day of life he had a series of "mild" convulsions, some generalized, others left-sided. Serum calcium was 6 mg. per cent, and he responded satisfactorily to calcium therapy. At this time an intracranial tap yielded an unknown quantity of blood-tinged fluid. He had been kept in the hospital for the first 2 months of life, but had no further convulsions, according to the mother. Dentition occurred at the usual time. During early infancy he developed a tremor of the head and a lateral nystagmus. Although he gained weight satisfactorily, mental development was slow. He sat unaided at 15 months of age, talked at 2 years, toilet training was never completely accomplished, and his speech remained somewhat dysarthric. At the age of 6½ years he was sent to school but did poorly, requiring much care with personal habits, and withdrawal from school was advised.

At the age of 7 he had a series of 3 generalized convulsions, characterized by tonic and clonic movements, carpopedal spasm, and postictal drowsiness. At about this same time he began to complain of headache.

Throughout childhood he has had nystagmus and head tremor, awkwardness of arm and leg movements, and peculiar "wringing" movements of his hands. Dental decay was noted in late infancy.

It is of interest that the mother had complete dentures by the age of 15, and that her fingernails are very thin and brittle. There are 3 siblings, 1 of whom suffers from moderately severe dental caries. None shows enamel hypoplasia. There is a strong history of dental caries in the mother's side of the family. The maternal grandmother has been in a mental institution on several occasions.

On examination, the patient weighed 25 kg. and was 128 cm. tall (50 per cent percentile for each). Lateral nystagmus and head nodding were noted. There was a generalized papular skin eruption. The optic lens was clear and the hair normal as to amount and texture. There were no abnormal neurologic signs. Chvostek's sign was present.

Laboratory studies revealed the following: serum calcium ranged between 6.2 and 8.8 mg. per cent, serum phosphorus, between 5.6 and 7.0 mg. per cent, and alkaline phosphatase was 9.5 units. Roentgenograms of the skull and cervical vertebrae were normal. Electroencephalogram revealed a paroxysmally slow rhythm. The intelligence quotient was 90. Dental examination at the age of 9 years revealed moderate caries but no enamel hypoplasia. Results of other studies revealed a normal bone age, and normal values for serum protein and nonprotein nitrogen. Intravenous pyelography showed slight kinking at the right ureteropelvic junction. Tests for syphilis were negative.

Treatment with vitamin D and oral calcium has produced a satisfactory response. Head nodding and nystagmus markedly lessened, movements became less awkward, and he is now doing well in school.

Case V. Judith F., a white female born October 30, 1940, was first seen at the age of four years. At the time of delivery (breech presentation) she was cyanotic, and was given a blood transfusion. She quickly recovered from this episode (the nature of which is not clear) and thrived as a young infant. Mental and physical development progressed normally. There is note of caries during infancy. At the age of 1 year it was observed that her voice was deep. She has always had some difficulty in swallowing liquids, although she handled solid foods well. At the age of $2\frac{1}{2}$ years she began to have episodes of facial cyanosis, facial grimacing, and would stumble. These attacks began in the spring of the year, and would often be precipitated by exercise. Shortly afterwards, she experienced her first convulsion, described as follows: prefaced by a loud shrill scream, the attack would begin with staring, generalized rigidity, backward fall, inspiratory and expiratory stridor, frothing and cyanosis. Shortly generalized clonic movements would begin, and these would last 4 to 5 minutes. She would then regain consciousness and, except for some residual spasticity, there were no postictal symptoms. The convulsions continued at irregular intervals for the next $1\frac{1}{2}$ years, being more frequent during the winter and spring months. A trial of phenobarbital and dilantin had no effect. During the interseizure periods she appeared normal except for frequent complaints of muscle tightness and a tendency to stumble when playing actively.

The patient has a younger brother who was diagnosed as having hypoparathyroidism at the age of $3\frac{1}{2}$ years. Another brother is deaf as a result of meningitis. The mother has thin, brittle fingernails and calcification of the dental pulp, and the father is said to have many carious teeth. The paternal grandmother had complete dentures at the age of 18. Her 3 sisters suffered from excessive dental decay, and her brother had short incisors. A paternal uncle and aunt each had fainting spells in childhood.

On examination the patient appeared healthy. Her weight was 17.8 kg. and her height 102 cm. (75th and 50th percentiles, respectively). No abnormalities of the skin, hair, nail, eyes, or mouth were noted. Chvostek's and Trousseau's signs were present, but otherwise the neurologic examination was normal. Intelligence quotient was 91. Dental examination (at the age of 14 years) revealed severe caries, enamel hypoplasia, pulp calcification, and an increase in density of the lamina dura (FIGURE 5).

Laboratory examination included serum calcium of 5.8 mg. per cent, phosphorus 10 mg. per cent, and alkaline phosphatase 6.8 units. Roentgenograms of the skull were normal, and the long bones were of normal density. An electroencephalogram revealed a normal basic rhythm on which were superimposed occasional high-amplitude 3-per-second bursts.

Results of other tests were: normal intravenous pyelogram, bone age survey, and urea clearance; BMR was +25 per cent at age 6; and there were normal values for serum protein. At 1 point she was found to have orthostatic albuminuria.

The patient has been observed for the past 10 years. Considerable improvement occurred on treatment with oral calcium combined with A.T. 10 or vitamin D. She has continued to complain of occasional muscle cramps, but only 1 further convulsion has occurred, and that was in association with temporary discontinuance of medication.

Other observations of interest during the follow-up period were the following: definite signs of dental caries and enamel hypoplasia were noted at the age of 5 years, and at about the same time some loss of hair was noted. At the age of 12 years, only 21 teeth were present; a number of these were carious, and many were small and pointed. Intelligence quotient was 85 to 90. There was no evidence of cataracts. Electroencephalogram was abnormal, with bursts of both slow and fast activity. Roentgenograms of the skull were normal. Menarche occurred at 13½ years.

Case VI. Lane T., the younger brother of case V, was first seen at the age of 4 years (birth date October 12, 1946). Early infancy and childhood were uneventful. Dentition occurred at 6 months, and mental and physical development were normal. It should be remarked, however, that from early infancy (he was born 2 years after his sister's diagnosis was made) he had been given approximately 2500 units of vitamin D daily.

Symptoms were first noted at the age of 3½ years, in the spring of the year. These consisted of muscle cramps involving calf muscles, and walking on tiptoe with the foot partially supinated. Positive Chvostek's and Trousseau's signs were elicited by his family physician and, with the finding of a serum calcium value of 6.4 mg. per cent and phosphorus of 6.4 mg. per cent, treatment with vitamin D and oral calcium was started at once. There was no history of convulsions, changes in the skin and hair, or carpopedal spasm.

On examination, the patient appeared healthy. His weight was 16.1 kg. and his height 98.5 cm. (50th and 15th percentiles, respectively). The teeth showed no signs of decay. Eyes, skin, and mouth were unremarkable. Neurologic examination was negative, and Chvostek's sign could not be elicited on treatment at the time.

Laboratory findings included serum calcium 8.6 to 10.5 mg. per cent; phosphorus, 4.4 to 6.2 mg. per cent; alkaline phosphatase, 12 to 18 units. Roentgenograms of skull and long bones were normal. Electroencephalogram (taken at the age of 7) revealed a paroxysmal dysrhythmia. Values for serum protein and nonprotein nitrogen were normal, and roentgen studies included normal bone age and intravenous pyelogram.

During a follow-up period of 4 years the patient has done well, making good progress in school and in physical development. A positive Chvostek's sign can frequently be elicited. Dental examination at the age of 9 years revealed severe caries and enamel hypoplasia. Carpopedal spasm occurs if therapy is omitted. On 1 occasion, mild azotemia was noted, and since this disappeared on reduction in vitamin D dosage, it was interpreted as evidence of vitamin D intoxication. Urinalysis and intravenous pyelography have failed to disclose evidence of renal disease.

Case VII. Kathy W., a white female born February 14, 1948, has the following history. Birth and early infancy were normal except for frequent digestive upsets. At the age of 2 years she developed an infection of the mouth, finally diagnosed as moniliasis. Despite local measures, the infection failed to subside, and soon became widespread, involving the face, forehead, and anal region. At the age of 2½ years, the fingernails were noted to be brittle, and they soon became thickened, yellowish in color, and wrinkled. Later, similar changes were seen in the toenails. Deepening of the voice was noted at the age of 3 years and, a short time later, the hair began to fall out easily. Because of repeated ear infections, a tonsilectomy and an adenoidectomy were done at the age of 4 years. Dental decay became noticeable at the age of 4½ years. At this time she began to have attack of "asthma" characterized by laryngeal stridor and wheezing. She also complained of pain in the extremities and abdomen. Examination at this time revealed the above-mentioned lesions of the fingernails, intention tremor of the hands, signs of moniliasis in the mouth and at the corners of the lips, a whitish plaque on the left vocal cord, and a fine scaling rash on the cheeks (the other skin lesions had cleared by this time). Attacks of wheezing, dyspnea, and stridor, sometimes associated with vomiting and irritability, increased in intensity, so that she was readmitted to the hospital for study, at the age of 5 years. Carpopedal spasm was not observed.

Measurements of weight and height placed her in the 25 per cent percentile for each. Chvostek's sign was positive. She was quite agitated and irritable. Except for the above described lesions, there were no remarkable physical findings other than rather marked dental caries and hypoplasia. The optic lens was clear. Roentgenograms of the skull and long bones were normal. Oral cultures again revealed monilia. Serum calcium was 3.6 to 5.0 mg. per cent; serum phosphorus, 10.9 to 13.5 mg. per cent; alkaline phosphatase, 4.7 units; and urea nitrogen, 12 mg. per cent.

There is 1 sibling living and well. The mother and maternal grandmother suffer from extensive dental decay, the latter needed complete dentures by the age of 28. The grandmother also had cataracts in later life. The mother and brother show normal values for serum calcium, phosphorus, and phosphatase.

Treatment with vitamin D and calcium has produced a satisfactory response as far as the respiratory tract symptoms are concerned, and the mouth lesions have cleared. The fingernails remain unchanged. Unfortunately, she continued to lose her hair to the point of total alopecia. At this juncture, A.T. 10 was substituted but produced no improvement over that achieved from the use of vitamin D. Consequently this latter medication was resumed after several months. After about a year of total alopecia, regrowth of hair occurred and is now approaching normal as to length and distribution, though it is still dry and somewhat brittle.

Note: Since this paper was submitted for publication, a review of the salient features of hypoparathyroidism, with a report of 6 juvenile cases, has been published by Harrison,[23] and a note on increased width of the lamina dura by Hurwitz[24] has also appeared.

References

1. Albright, F. & E. C. Reifenstein, Jr. 1948. The Parathyroid Glands and Metabolic Bone Disease. Williams & Wilkins. Baltimore, Md.
2. Lachmann, A. 1941. Hypoparathyroidism in Denmark: clinical study. Acta Med. Scand. Suppl. **121:** 1.
3. Jordan, A. & A. R. Kelsall. 1951. Observations on a case of hypoparathyroidism. Arch. Internal Med. **87:** 242.
4. Steinberg, H. & B. R. Waldron. 1952. Idiopathic hypoparathyroidism: an analysis of 52 cases, including the report of a new case. Medicine. **31:** 133.
5. Walton, L. 1954. Neonatal tetany in two siblings: effect of maternal hyperparathyroidism. Pediatrics. **13:** 227.

6. Emerson, K., Jr., F. B. Walsh & J. E. Howard. 1941. Idiopathic hypoparathyroidism: report of two cases. Ann. Internal Med. **14:** 1256.

7. Papadatos, C. & R. Klein. 1954. Addison's disease in a boy with hypoparathyroidism. J. Clin. Endocrinol. and Metabolism. **14:** 653.

8. McLean, M. M. 1954. Chronic idiopathic hypoparathyroidism associated with moniliasis. Arch. Disease Childhood. **29:** 419.

8a. McGregor, M. E. & T. P. Whitehead. 1954. Pseudohypoparathyroidism. Arch. Disease Childhood. **29:** 398.

9. Talbot, N. B. 1952. Functional Endocrinology from Birth Through Adolescence. Harvard Univ. Press. Cambridge, Mass.

10. Barr, D. P., C. M. MacBryde & T. E. Sanders. 1938. Tetany with increased intracranial pressure and papilledema—results from treatment with dihydrotachysterol. Trans. Assoc. Am. Physicians. **53:** 227.

11. Sutphin, A., F. Albright & D. McCune. 1943. Five cases (three in siblings) of idiopathic hypoparathyroidism associated with moniliasis. J. Clin. Endocrinol. and Metabolism. **3:** 625.

12. Schour, I., S. B. Chandler & W. R. Tweedy. 1937. Changes in the teeth following parathyroidectomy. Am. J. Pathol. **13:** 945, 971.

13. Leifer, E. & W. Hollander. 1953. Idiopathic hypoparathyroidism and chronic adrenal insufficiency. J. Clin. Endocrinol. and Metabolism. **13:** 1264.

14. Ostertag, B. 1930. Die an bestimmte Lokalisation gebundenen Konkremente des Zentralnervensystems und ihre Beziehung zur "Verkalkung intracerebraler Gefässe" beigewissen endokrinen Erkrankungen. Virchow's. Arch. **275:** 828.

15. Moehlig, R. C. & A. L. Steinbach. 1954. Cortisone interference with calcium therapy in hypoparathyroidism. J. Am. Med. Assoc. **154:** 42.

16. Goldman, R., J. L. Reynolds, H. R. Cummings & S. H. Bassett. 1952. Familial hypoparathyroidism: report of a case. J. Am. Med. Assoc. **150:** 1104.

17. Warkany, J. & A. M. DiGeorge. 1954. *In* Nelson's Textbook of Pediatrics. W. A. Saunders. Philadelphia, Pa.

18. Craig, J. M., L. H. Schiff & J. E. Boone. 1955. Chronic moniliasis associated with Addison's disease. Am. J. Diseases Children. **89:** 669.

19. Schulman, J. L. & H. Ratner. 1955. Idiopathic hypoparathyroidism with bony demineralization and cardiac decompensation. Pediatrics. **16:** 848.

20. Reisner, D. J. & R. M. Ellsworth. 1955. Coexistent idiopathic hypoparathyroidism and pernicious anemia in a young girl: case report. Ann. Internal Med. **43:** 1116.

21. Himsworth, H. P. & M. Maizels. 1940. Vitamins D_2 and D_3 and A. T. 10 in congenital thyroid and parathyroid deficiency. Lancet. **1940**(i): 959.

22. Simpson, J. A. 1954. Dermatologicial changes in hypocalcemia. Brit. J. Dermatol. **66:** 1.

23. Harrison, H. E. 1956. Idiopathic hypoparathyroidism. Pediatrics. **17:** 442.

24. Hurwitz, L. J. 1956. Spontaneous hypoparathyroidism with megaloblastic anemia. Lancet. **1956**(i): 234.

STUDIES ON PARATHYROID EXTRACT IN NORMAL SUBJECTS AND A PATIENT WITH PSEUDOHYPOPARATHYROIDISM*

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Over a decade ago Albright[1] described 3 patients with hypocalcemia and hyperphosphatemia. In these cases, there was no alteration in urinary phosphorus excretion following intravenously administered parathyroid extract. He postulated that these individuals had end-organ refractoriness (presumably renal) to endogenous, as well as to exogenously supplied, parathyroid hormone. The name "pseudohypoparathyroidism" or the "Seabright Bantam Syndrome" was suggested for this abnormality. The former designation seems to have been accepted by subsequent observers.

We recently had an opportunity to study a patient with this syndrome. We were particularly interested in identifying the nature of the renal lesion and in investigating the effects of exogenous parathyroid hormone on phosphate excretion and on the level of phosphorus in the serum. Since the effects of parathyroid extract on renal phosphorus excretion in normal subjects is not clear, it seemed important to examine the renal phosphorus response of a group of normal subjects under conditions identical with those to be used for study of the patient with pseudohypoparathyroidism.

Subcutaneously administered inulin was used to determine glomerular filtration rate. A blood level of between 25 and 50 mg. per cent of inulin was uniformly achieved. Periods of urine collection varied from 20 to 40 minutes, and a 90-minute equilibration period was always allowed. The same lot number of parathyroid extract was used for both the normal subjects and the patient with pseudohypoparathyroidism. No fluid was administered intravenously during the experimental periods. Glomerular filtration rate and phosphorus clearance studies were done before, during, and immediately following the administration of parathyroid extract. Levels of calcium and phosphorus were determined in concommitantly obtained serum, and phosphorus was measured in the urine. The results of these studies are the basis of the data to be presented.

Figure 1 shows 1 of the consequences of the administration of parathyroid extract to normal subjects. An increase in phosphaturia that occurred during the 2 hours following the intravenous administration of parathyroid extract was prompt, and reached its maximum in about 1 hour. The administration of 200 units of parathyroid extract resulted in a rise in urinary phosphorus excretion from 0.10 mg. min. to 0.65 mg. min., while a smaller dosage showed a similar response but one somewhat less in magnitude. The increase in phosphorus excretion following the administration of parathyroid extract intravenously is well known as the "Ellsworth-Howard Test,"[2] and is reported here merely to show the efficacy of this lot of parathyroid extract. Numerous investigators have attempted to explain the characteristic response (increase in

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* The parathyroid extract used in these studies was obtained from Eli Lily and Company, Indianapolis, Ind.

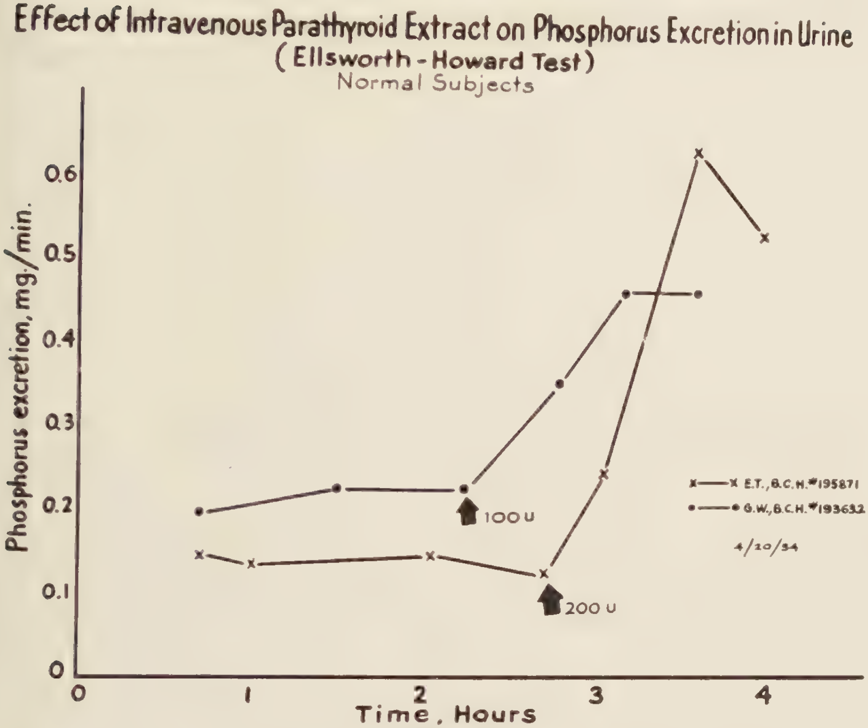

FIGURE 1

urinary phosphorus to intravenously administered parathyroid extract. It has been demonstrated by Handler, using dogs, and later by Klein,[4] that when parathyroid extract is administered intravenously, there is an increase in glomerular filtration rate. With this change in filtration, there is an increase in phosphate load. The effect of parathyroid hormone on urinary phosphorus was therefore considered by these investigators to be a consequence of an increased phosphate load on the renal tubules. This by itself should not have affected urinary phosphorus unless the new load was beyond the capacity of tubular reabsorption. Handler also reported that changes in the glomerular filtration rate resulting from the injection of parathyroid extract fails to appear in dogs if the hormone is given subcutaneously. When we repeated his experiments on a normal human, using the intramuscular route, we obtained results similar to those found by Handler for the dog. The intravenous administration of 200 units of parathyroid extract to 2 normal subjects on 4 occasions produced a 34 per cent increase in glomerular filtration rate over the preinjection level. The intramuscular route of hormone administration did not affect the glomerular filtration rate, yet the magnitude of phosphaturia induced by these 2 routes of hormone administration were comparable.

TABLE 1 summarizes our measurements of glomerular filtration rate and tubular phosphorus reabsorption in response to intravenous and intramuscular para-

TABLE 1

PARATHYROID EXTRACT: EFFECTS ON RENAL FUNCTION

Route of administration	Percent Change		
	Glomerular G.F.R. ml./min.	Tubular P reabsorbed mg./min.	Tubular/Glomerular P reabsorbed mg./100 ml. G.F.R.
I.V.	+34	−16	−37
	+33	−13	−33
	+30	−10	−32
I.M.	−5	−41	−38
	0	−23	−23
	+2	−17	−19

thyroid extract in normal subjects. It re-emphasizes the absence of an effect on glomerular filtration rate when the hormone is administered intramuscularly. There exists a consistent decrease in phosphorus reabsorption, and the intramuscular effect on phosphaturia seems to be greater than that of the intravenous effect. If inulin can be accepted as a measure of glomerular filtration rate, then there can be no question that tubular reabsorption substantially decreases when parathyroid extract is given intramuscularly. It can be argued, however, that inulin may not measure glomerular filtration rate sensitively enough to determine the true filtered load of phosphate. In that case, a slight change in glomerular filtration rate could account for an increased load to the tubules and, possibly, produce increased phosphaturia if the new load were greater than the maximum tubular reabsorption for phosphorus. The constancy of the glomerular filtration rate should support the reliability placed on the inulin method. When the tubular response is expressed in terms of load (glomerular filtration rate) and compared to results obtained when there is no change in glomerular filtration rate, it seems clear that there is an effect on tubular phosphorus reabsorption.

FIGURE 2 shows the effect of parathyroid extract on glomerular filtration rate and phosphorus excretion in the urine of a patient with pseudohypoparathyroidism. The scale used on this chart is similar to that used in plotting the data of our normal subjects.

The parathyroid extract had no effect on phosphaturia. This was anticipated on the basis of work by several investigators.[1, 5, 7] It is obvious, however, that this patient did not respond, either with a rise in glomerular filtration rate or with an alteration in phosphate excretions to the intravenously administered hormone. This test was repeated 3 times, with a similar lack of response.

We noted, during the course of these studies of normal subjects after hormone injection, that the serum phosphorus levels fell and that the serum calcium levels rose. This was the anticipated result. In the patient with pseudohypoparathyroidisim, however, both elements rose in concentration. Since this was a consistent finding in our patient, we sought similar data from previously reported patients.[1, 3, 5, 6] FIGURE 3 summarizes these collected data.

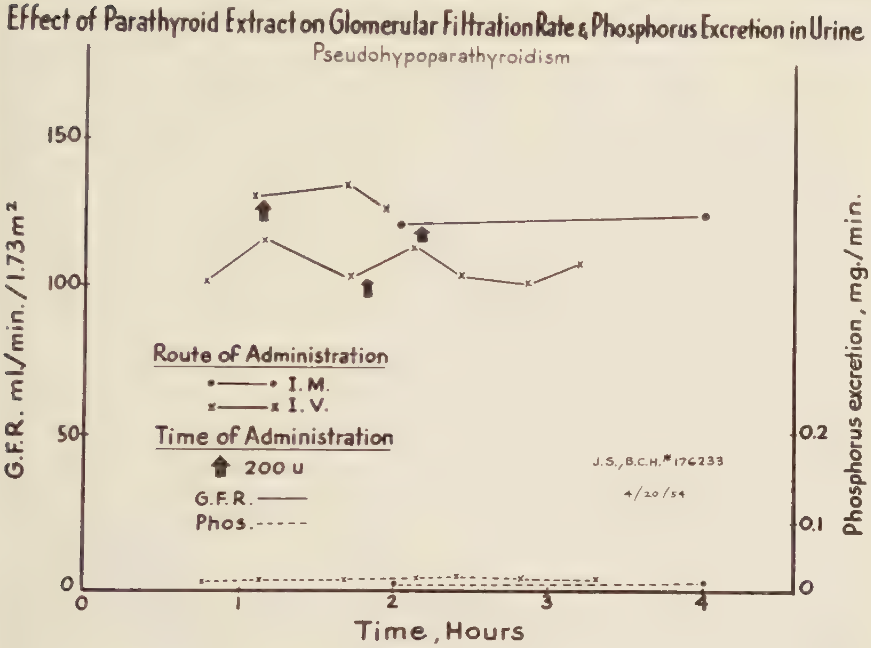

FIGURE 2

In general, patients with pseudohypoparathyroidism show a significant rise in both phosphorus and calcium of the serum, even though it has been demonstrated that these patients do not have a renal response to parathyroid extract. This rise in serum phosphorus concentration would indicate a nonrenal response. If this conclusion is correct, and since the glomerular filtration rate is normal and the excretion of phosphorus by these patients appears to be minimal, the administration of parathyroid extract for a prolonged period of time should result in a significantly increased excretion of phosphorus in the urine. To test this hypothesis, we administered 200 units per day of parathyroid extract for 3 days to the patient with pseudohypoparathyroidism while she was on a constant diet. The data obtained are shown on FIGURE 3. In spite of the fact that 4 hours after parathyroid extract administration there was a rise in serum phosphorus, it can be seen that 24 hours after each succeeding dose of extract administration, there was a continuous fall in serum phosphorus. This can be explained in 1 of 2 ways: either by a redeposition of the liberated phosphorus in the body tissues or by an excretion of phosphorus by the kidney. During the control period before the administration of parathyroid extract, this patient excreted 200 mg. of phosphorus. On the 2nd and 3rd days of the study period, following the administration of the extract, this subject had double and triple increases of urine phosphorus. The excretion of phosphorus was minimal and does not explain entirely the fall in serum phosphorus concentration.

Unanimity of opinion among investigators concerning the renal effects of

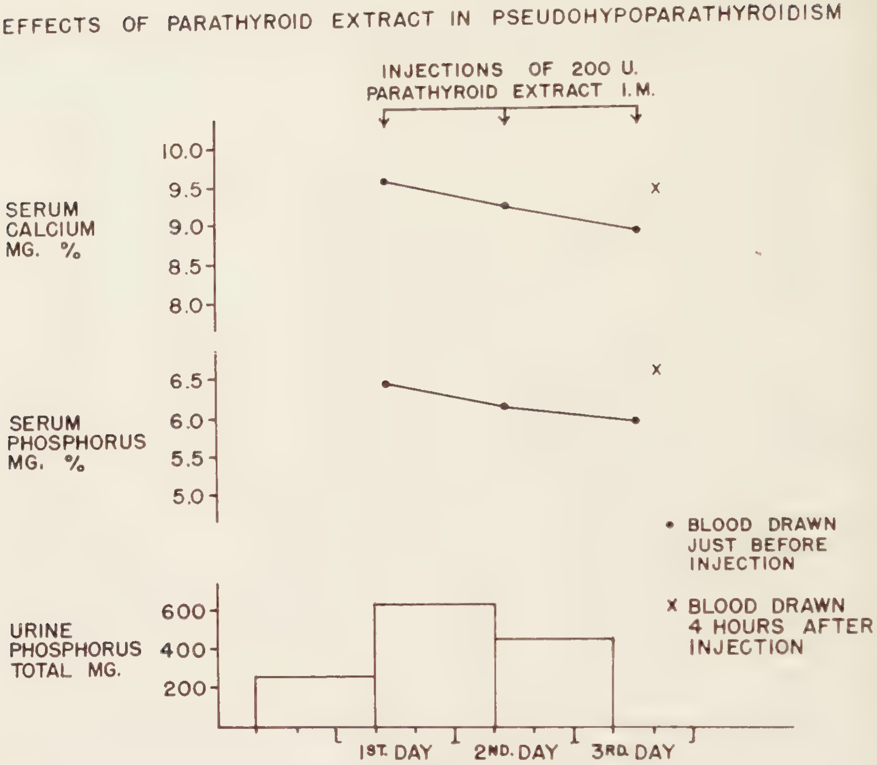

FIGURE 3

parathyroid extract administration has not been established. There are those who believe that the kidney does not respond to the hormone. Marion Fay and her associates,[10] who studied phosphate creatinine ratios in normal, parathyroid-treated, and parathyroidectomized dogs, suggest that a lack or an excess of the hormone showed no apparent effect upon renal phosphate excretion. Jahan and Pitts[11] found that the administration of the hormone in large quantities did not change the capacity of the renal tubules to reabsorb phosphate. Other investigators support the concept that the primary renal effect following parathyroid extract administration is a depression of the reabsorption of phosphate by the renal tubules, resulting in phosphaturia. Harrison and Harrison[12] studied the renal phosphorus reabsorption in dogs, subsequent to the subcutaneous administration of the hormone, and concluded that there is a considerable decrease in the rate of reabsorption of phosphate by the renal tubules. Cargill and Witham,[13] using the intravenous administration of parathyroid extract in man, found the increased excretion of phosphate to be due entirely to decreased tubular reabsorption. McCrory *et al.*,[14] in administering 15 units of parathyroid extract to newborn infants, attributed the observed phosphaturia to significant decreases in the rate of phosphate reabsorption.

One of the effects following parathyroid extract administration, observed by several investigators, has been a significant increase in the glomerular filtration rate. Phosphaturia occurring under such circumstances is said not to be accompanied by a decrease in tubular reabsorption of phosphate. Michie and Shorey,[15] who studied a patient with hypoparathyroidism, interpreted the increase in phosphaturia to a slight increase in the glomerular filtration rate. Handler *et al.*[3] showed that dogs given parathyroid extract intravenously responded with a rise in glomerular filtration rate and renal plasma flow that resulted in an increase in phosphaturia. Subcutaneous injection produced smaller hemodynamic changes, but a consistent decline in tubular reabsorption accounted for the phosphaturia in each incidence. Hiatt and Thompson[16] studied the effects of intravenously administered parathyroid extract (200 to 1000 U.S.P. units) in man during an infusion of buffered sodium phosphate permitting calculation of maximum tubular reabsorption using 6 normal and 2 hypoparathyroid subjects, and found a prompt rise in urinary phosphate in 28 studies. No immediately measurable change in maximum tubular reabsorption for phosphate was observed in 11 studies, and the phosphaturia was attributed to increased glomerular filtration rate. These investigators also gave 1600 to 4000 units of the extract over 48 to 96 hours, and produced a 20 to 60 per cent fall in the maximum reabsorption for phosphate in all normal and hypoparathyroid subjects.

Crawford *et al.*[17] suggest a relationship between the ratio of phosphorus reabsorbed by the tubules to phosphorus in glomerular filtrate (T.R.P./G.F.P.) and parathyroid gland activity. Although elevation of the glomerular filtration rate occurred regularly following parathyroid extract administration, variations in urinary phosphorus excretion observed were not believed to be a direct function of the filtered phosphorus.

Observed differences in response to parathyroid extract administration obviously may be due to heterogeneous mixtures of parathyroid extract. It has not been established whether or not the effect observed upon glomerular filtration is due to an active principle or to a nonspecific element in the extract administered. It has become increasingly clear that the time, route, and quantity of administration of the parathyroid extract may be factors determining the response. Recently Goldman and Bassett[18] studied the relationship of phosphate clearance to the general level of renal function, and tested the response to exogenous parathyroid hormone at all gradations of renal impairment. These investigators showed very clearly that the administration of parathyroid extract caused an increase in urinary phosphate that was apparently due to both an increase in the glomerular filtration rate and a decrease in tubular reabsorption. The responsiveness to parathyroid extract was lost in renal disease when the glomerular filtration rate was reduced below 10 ml. per minute. Our data support those investigators who accept an effect of parathyroid extract upon both the glomerular filtration rate and tubular reabsorption of phosphate. Whether or not the lack of exogenous-hormone response on glomerular filtration in the patient with pseudohypoparathyroidism is specific cannot be determined from data on this 1 child. The data also support those who hold that parathyroid extract also affects nonrenal tissue, possibly bone.[19, 20]

These data furthermore suggest the following conclusions:

(1) Depending upon the route of administration, parathyroid extract ex-

hibits effects on glomerular filtration rate and/or tubular reabsorption of phosphorus.

(2) The intravenous administration of parathyroid extract to a normal individual may cause a rise in glomerular filtration rate. Phosphaturia can occur independently of such an increase in glomerular filtration rate.

(3) Our patient with pseudohypoparathyroidism exhibits a failure to achieve the increase in glomerular filtration rate found in our normal controls following administration of parathyroid extract. It is emphasized that we report only a single case.

4. The data support the belief that part of the effect of parathyroid extract must be upon nonrenal tissue, since patients without renal response nevertheless showed increases in serum calcium and phosphorus.

References

1. Albright, F., C. H. Burnett, P. H. Smith & W. Parson. 1942. Pseudo-hypoparathyroidism—an example of Seabright Bantam Syndrome. Endocrinology. **30:** 922.
2. Ellsworth, R. & J. E. Howard. 1934. Studies on the physiology of the parathyroid glands. Bull. Johns Hopkins Hosp. **55:** 296.
3. Handler, P., D. V. Cohn & W. J. A. DeMaria. 1951. Effect of parathyroid extract on renal excretion of phosphate. Am. J. Physiol. **165:** 434.
4. Klein, R. & R. C. Gow. 1953. Interaction of parathyroid hormone and vitamin D on the renal excretion of phosphate. J. Clin. Endocrinol. and Metabolism. **13:** 271.
5. Alexander, S. B. & H. St. George Tucker, Jr. 1949. Pseudohypoparathyroidism—report of a case with late manifestations. J. Clin. Endocrinol. **9:** 862.
6. Lowe, C. U., A. J. Ellinger, W. S. Wright & H. M. Stauffer. 1950. Pseudohypoparathyroidism (the Seabright Bantam syndrome). J. Pediat. **36:** 1.
7. Elrick, H., F. Albright, F. C. Bartter, A. P. Forbes & J. Reeves. 1950. Further studies on pseudohypoparathyroidism: report of four new cases. Acta Endocrinol. **5:** 199.
8. Peterman, M. G. & G. L. Garvey. 1949. Pseudohypoparathyroidism: case report. Pediatrics **4:** 790.
9. Reynolds, T. B., G. Jacobson, H. A. Edmondson, H. E. Martin & C. H. Nelson. 1952. Pseudohypoparathyroidism. Report of a case showing bony demineralization. J. Clin. Endocrinol. and Metabolism. **12:** 560.
10. Fay, M., V. G. Behrmann & D. M. Bruck. 1942. The parathyroids and the clearance of inorganic phosphate. Am. J. Physiol. **136:** 716.
11. Jahan, I. & R. F. Pitts. 1948. Effect of parathyroid on renal tubular reabsorption of phosphate and calcium. Am. J. Physiol. **155:** 42.
12. Harrison, H. E. & H. C. Harrison. 1941. The renal excretion of inorganic phosphate in relation to the action of vitamin D and parathyroid hormone. J. Clin. Invest. **20:** 47.
13. Cargill, W. H. & A. C. Witham. 1949. Parathormone and the tubular reabsorption of glucose and phosphate in man. Federation Proc. **8:** 21.
14. McCrory, W. W., C. W. Forman, H. McNamara & H. L. Barnett. 1952. Renal excretion of inorganic phosphate in newborn infants. J. Clin. Invest. **31:** 357.
15. Michie, A. J. & J. M. Shorey. 1950. Parathyroid extract hyperphosphaturia in hypoparathyroidism. Federation Proc. **9:** 88.
16. Hiatt, H. H. & D. D. Thompson. 1955. The effects of parathyroid extract on renal function in man. J. Clin. Invest. **34:** 940.
17. Crawford, J. D., M. M. Osborne, Jr., N. B. Talbot, M. L. Terry & M. F. Morrill. 1950. The parathyroid glands and phosphorus homeostasis. J. Clin. Invest. **29:** 1448.
18. Goldman, R. & S. H. Bassett. 1954. Phosphorus excretion in renal failure. J. Clin. Invest. **33:** 1623.
19. Selye, H. 1932. Stimulation of new bone formation with parathyroid extract and irradiated ergosterol. Endocrinology. **16:** 547.
20. Collip, J. B., L. I. Pugsley, H. Selye & D. L. Thomson. 1934. Observation concerning the mechanism of parathyroid hormone action. Brit. J. Exptl. Pathol. **15:** 335.

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